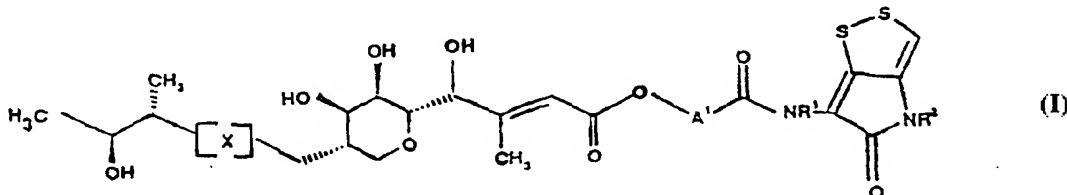


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(54) Title: THIOMARINOL DERIVATIVES, PROCESS AND INTERMEDIATES FOR THEIR PREPARATION AND THEIR USE AS MICROBICIDES AND HERBICIDES



(57) Abstract

Ester derivatives of 4-hydroxymonic acid of formula (I) comprising a terminal pyrrothine moiety have useful anti-bacterial, anti-fungal and herbicidal properties. Several intermediates have been claimed as well.

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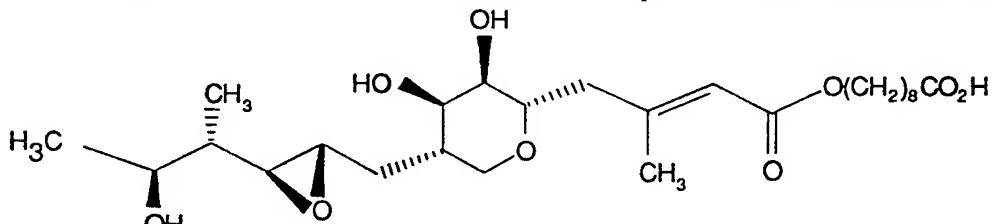
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THIOMARINOL DERIVATIVES, PROCESS AND INTERMEDIATES FOR THEIR PREPARATION AND THEIR USE AS MICROBICIDES AND HERBICIDES

5 This invention relates to a novel class of compounds having antibacterial, antimycoplasmal and antifungal activity, to processes for their preparation and to their use in human and veterinary medicine, and also to intermediates for use in the preparation of such compounds. These compounds also have herbicidal activity and therefore will be of use in agriculture.

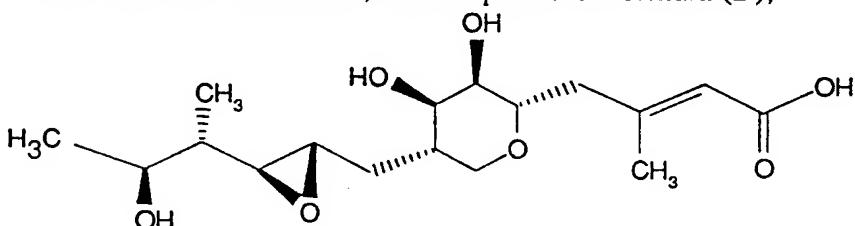
10 The microorganism *Pseudomonas fluorescens* produces three closely related tetrahydropyranyl compounds known as pseudomonic acids A, B and C which are of interest on account of their antibacterial properties.

Pseudomonic acid A (now known as mupirocin) has the structure (A):



(A)

It is an ester of monic acid, the compound of formula (B);



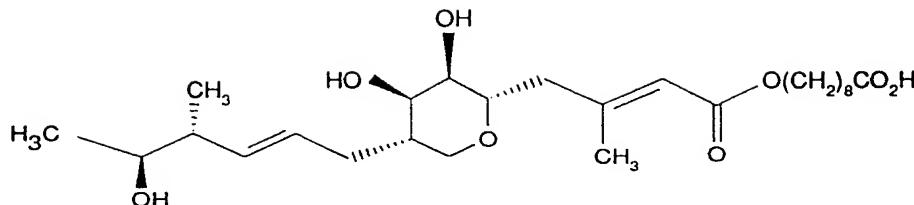
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(B)

in which the ester forming radical is derived from 9-hydroxynonanoic acid.

Pseudomonic acid A exhibits good anti-bacterial activity, mainly against Gram-positive bacteria, but also against some Gram-negative bacteria such as *Haemophilus influenzae* and *Moraxella catarrhalis*. It acts as selective reversible inhibitor of bacterial *iso*-leucyl t-RNA synthetase, thereby inhibiting bacterial protein synthesis. It also has anti-mycoplasma and anti-fungal activity (see Merck Index, 11th edn, 1989, 993 and references therein and EP 0 251 434-A). The compound is marketed by SmithKline Beecham under the trade mark Bactroban, as a topical formulation. Systematic use is precluded by a rapid metabolism to monic acid, which is inactive. More recently, it has been disclosed that esters of monic acid also have useful herbicidal activity (WO 93/19599, Zeneca Ltd).

Pseudomonic acid C has the structure (C):

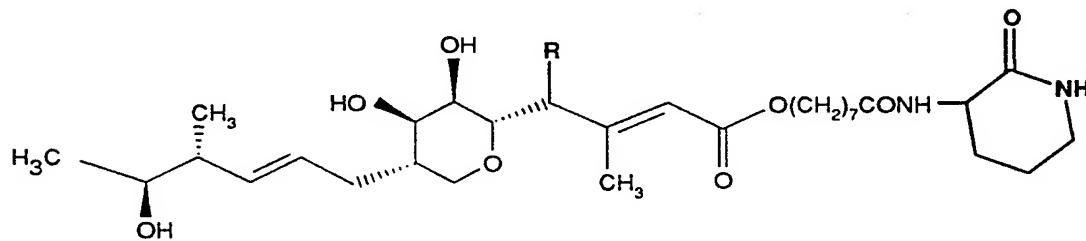


(C)

(EP 0 003 069, Beecham Group Ltd) and is distinguished from pseudomonic acid A by the presence of a C10-C11 *trans*-double bond.

5 More recently, there have been reports of compounds produced by marine
microrganisms which are closely related to pseudomonic acid and have antibacterial
activity. These compounds have the C10-C11 *trans*-double bond of the "C" series
and are characterised by the presence of a hydroxyl substituent at C-4. In addition,
the ester forming radical is derivable from 8-hydroxyoctanoic acid in which the
10 carboxy terminal is present as an amide formed from an amine containing a
heterocyclic moiety.

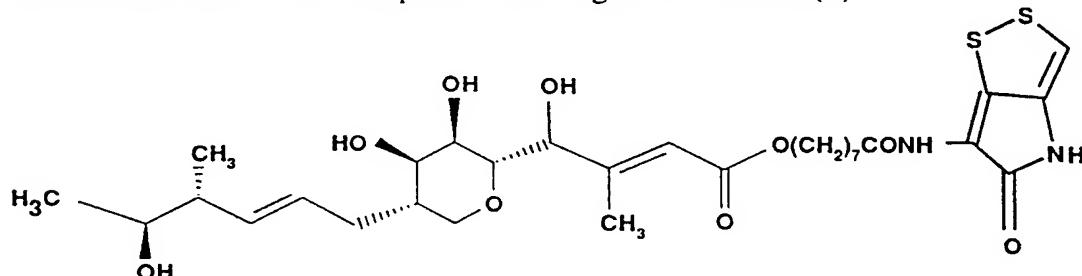
The compound of formula (D):



(D)

15 in which R is hydrogen or hydroxyl is produced by an *Alteromonas* species associated
with a marine sponge (Stierle D B and Stierle A A, 200th National Meeting of ACS,
Washington DC, Aug 26-31, 1990 and *Experientia*, 1992, **48**, 1165). The
stereochemistry of the C-4 hydroxyl was inferred to be β -, based on spectroscopic
20 studies of a cyclised derivative.

In addition, a further compound, named thiomarinol, is produced by
Alteromonas rava. This compound has the general formula (E):



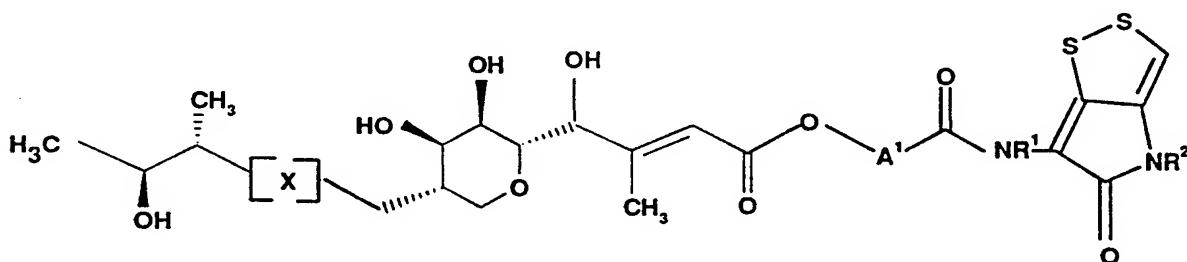
(E)

25 the stereochemistry being the same as that in pseudomonic acid C at each of the

common chiral centres. The stereochemistry of the 4-hydroxyl substituent, however, remains undefined (EP 0 512 824-A1, Sankyo Co Ltd and Shiozawa et al, J Antibiotics, 1993(12), 46, 1834-1842). Thiomarinol is said to possess good anti-bacterial activity against both Gram-positive and Gram-negative organisms, as well as 5 being active against mycoplasma. The amine forming the terminal amide is a pyrrothine, in particular holothin. The acetamides thereof include the known anti-bacterial compounds thiolutin (Merck Index, 11th edn, 1989, 1471) and holomycin (Merck Index, 11th edn, 1989, 747). Thiolutin also has anti-fungal activity.

10 We have now found that further pyrrothine derivatives have useful therapeutic properties.

Accordingly, the present invention provides a compound of formula (I):

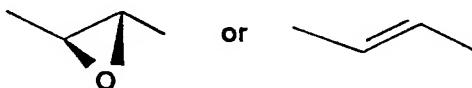


15

(I)

in which A^1 is a group of atoms for linking $C(O)O$ with $CONR^1$;
 R^1 and R^2 , which may be the same or different, is each selected from hydrogen or
 (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl, (C_{2-6}) alkenyl, aryl, aryl($C_{1-4})$ alkyl or heterocyclyl,
each of which may be optionally substituted;

20 X is an epoxy moiety or an E-double bond moiety:



and excluding the compound named "thiomarinol".

25 Compounds of formula (I) have improved anti-bacterial properties both in terms of absolute potency and an enhanced spectrum, compared to pseudomonic acids A and C.

30 The linking group of atoms A^1 comprises one or more carbon atoms which could include carbon atoms in a carbocyclic, for instance, an aryl, ring and/or heteroatoms, for instance nitrogen, sulphur and oxygen, which could include heteroatoms in a heterocyclic ring.

Suitable values for A^1 include the following:

$C(R^3)(R^4)$; $[C(R^3)(R^4)]_m A^2$; $[C(R^3)(R^4)]_m A^2 A^3$; $[C(R^3)(R^4)]_m A^3$; and $[C(R^3)(R^4)]_m A^3 A^2$;

in which:

m is 0 or 1 (such when m is 0, $[C(R^3)(R^4)]_m$ represents a bond);

5 R^3 and R^4 , which may be the same or different, is each selected from hydrogen or $(C_{1-6})alkyl$;

A^2 is a $(C_{3-7})cycloalkylene$ group, an optionally substituted aryl group, preferably phenylene, or an optionally substituted heterocyclyl group; and

A^3 is a polymethylene chain having between 1 and 20 carbon atoms,

10 preferably 4 and 9 carbon atoms, which chain may be optionally substituted, for instance by a $(C_{1-6})alkyl$ group, and which chain may be optionally interrupted at one or more places by a moiety M in which:

M is a chain of one or more atoms for linking two polymethylene chains and which may be the same or different if there is more than one interruption.

15 Suitable values for M include a heteroatom selected from oxygen, sulphur or nitrogen, preferably oxygen; a $(C_{3-7})cycloalkylene$ group; a carbon-carbon double bond; a carbon-carbon triple bond; CO ; $OC(O)$; $C(O)O$; $NRCO$; $C(O)NR$; $NRCONR$; $NRC(O)O$; $OC(O)NR$; SO_2NR ; $NRSO_2$; $CONRSO_2$; SO_2NRCO and phenoxy; in which R is hydrogen or $(C_{1-6})alkyl$.

20 Suitably, R^1 is hydrogen or $(C_{1-6})alkyl$, preferably hydrogen.

Suitably, R^2 is hydrogen or $(C_{1-6})alkyl$, for instance methyl, preferably hydrogen.

Suitably, A^1 is an optionally substituted polymethylene chain $(CH_2)_n$ in which n is an integer from 1 to 10, preferably from 5 to 9, more preferably 6, 7 or 8.

25 Suitable substituents include $(C_{1-6})alkyl$, in particular on the α -carbon (carbon attached to carboxy).

It will be readily appreciated by the skilled person that the carbon adjacent to the tetrahydropyran ring and bearing a hydroxyl group is a chiral centre and may have either absolute configuration. The present invention covers both possibilities.

30 Preferably, the hydroxyl group has the same configuration as that in thiomarinol.

Suitable substituents for a $(C_{1-6})alkyl$, $(C_{3-7})cycloalkyl$ or $(C_{2-6})alkenyl$ group or a polyalkylene chain include for example, halogen, cyano, azido, nitro, carboxy, $(C_{1-6})alkoxycarbonyl$, carbamoyl, mono- or di- $(C_{1-6})alkylcarbamoyl$, sulphonyl, sulphamoyl, mono- or di- $(C_{1-6})alkylsulphamoyl$, amino, mono- or

35 di- $(C_{1-6})alkylamino$, acylamino, ureido, $(C_{1-6})alkoxycarbonylamino$, 2,2,2-trichloroethoxycarbonylamino, aryl, heterocyclyl, hydroxy, $(C_{1-6})alkoxy$, acyloxy, oxo, acyl, 2-thenoyl, $(C_{1-6})alkylthio$, $(C_{1-6})alkylsulphinyl$, $(C_{1-6})alkylsulphonyl$, hydroxyimino, $(C_{1-6})alkoxyimino$, hydrazino, hydrazono,

benzohydroximoyl, guanidino, amidino and iminoalkylamino.

When used herein, the term 'aryl' includes, unless otherwise defined, phenyl or naphthyl optionally substituted with up to five, preferably up to three substituents.

Suitable substituents for an aryl group include, for example, halogen, cyano,

5 (C₁₋₆)alkyl, phenyl, (C₁₋₆)alkoxy, halo(C₁₋₆)alkyl, hydroxy, amino, mono- or di-(C₁₋₆)alkylamino, acylamino, nitro, carboxy, (C₁₋₆)alkoxycarbonyl, (C₁₋₆)-alkoxycarbonyl(C₁₋₆)alkyl, (C₁₋₆)alkylcarbonyloxy, (C₁₋₆)alkylthio, (C₁₋₆)alkylsulphinyl, (C₁₋₆)alkylsulphonyl, sulphamoyl, mono- or di-(C₁₋₆)alkylsulphamoyl, carbamoyl, and mono- or di-(C₁₋₆)alkylcarbamoyl.

10 When used herein, the term 'heterocyclyl' includes aromatic and non-aromatic single or fused rings comprising up to four heteroatoms in the ring selected from oxygen, nitrogen and sulphur and optionally substituted with up to three substituents. Suitably the heterocyclic ring comprises from 4 to 7, preferably 5 to 6, ring atoms. A fused heterocyclic ring system may include carbocyclic rings and need only include 15 one heterocyclic ring. Suitable substituents for a heterocyclyl group include those hereinbefore defined for an aryl group, as well as oxo.

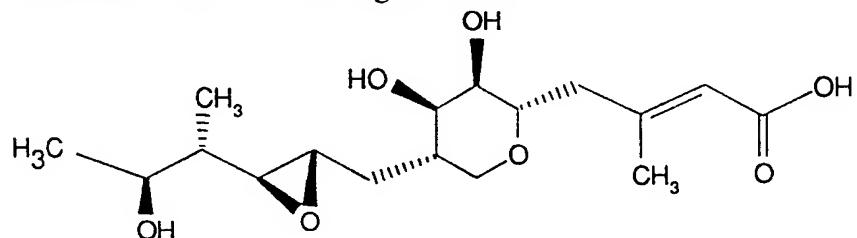
When used herein, the term 'halogen' refers to fluorine, chlorine, bromine or iodine.

20 Since the compounds of formula (I) of the present invention are intended for use in pharmaceutical compositions, it will be understood that they are each provided in substantially pure form, for example at least 50% pure, more suitably at least 75% pure and preferably at least 95% pure (% are on a wt/wt basis). Impure preparations of the compounds of formula (I) may be used for preparing the more pure forms used in the pharmaceutical compositions. Although the purity of intermediate compounds 25 of the present invention is less critical, it will be readily understood that the substantially pure form is preferred as for the compounds of formula (I). Preferably, whenever possible, the compounds of the present invention are obtained in crystalline form.

When some of the compounds of this invention are allowed to crystallise, or 30 are recrystallised, from organic solvents. solvent of crystallisation may be present in the crystalline product. This invention includes within its scope such solvates. Similarly, some of the compounds of this invention may be crystallised or recrystallised from solvents containing water which may lead to the formation of 35 hydrated products. This invention includes within its scope stoichiometric hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

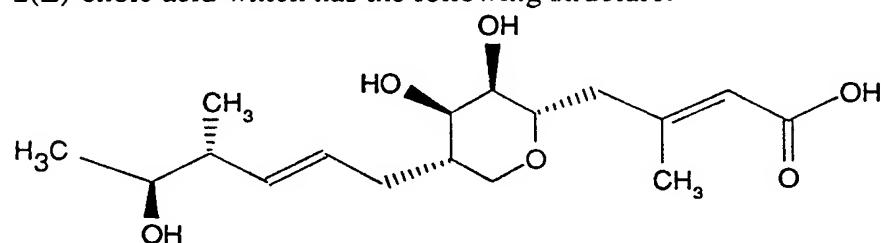
It will be readily appreciated that compounds of formula (I) are esters of 4-hydroxy analogues of either monic acid A or monic acid C. Monic acid A is the

name given to the compound 4-[(2S,3R,4R,5S)-5-[(2S,3S,4S,5S)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxytetrahydropyran-2-yl]-3-methyl-but-(E)-enoic acid which has the following structure:



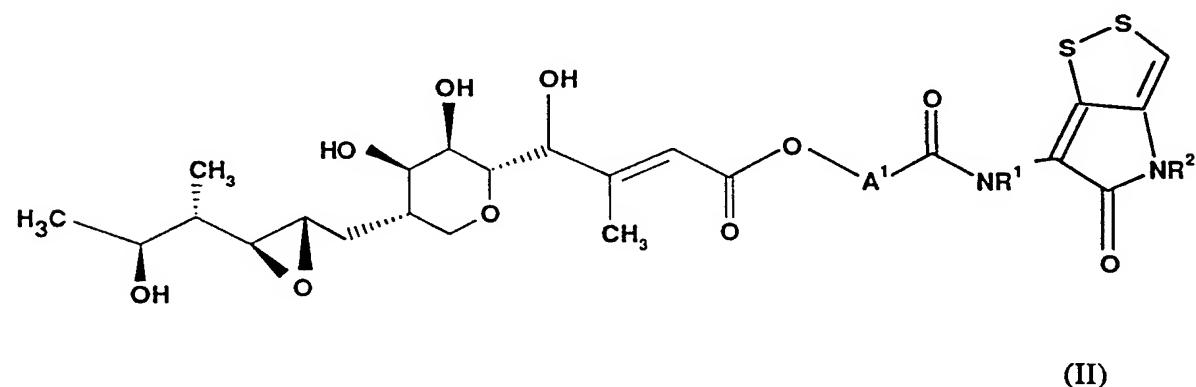
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Monic acid C is the name given to the compound 4-[(2S,3R,4R,5S)-5-[(4R,5S)-5-hydroxy-4-methylhex-2(E)-enyl]-3,4-dihydroxytetrahydropyran-2-yl]-3-methylbut-2(E)-enoic acid which has the following structure:



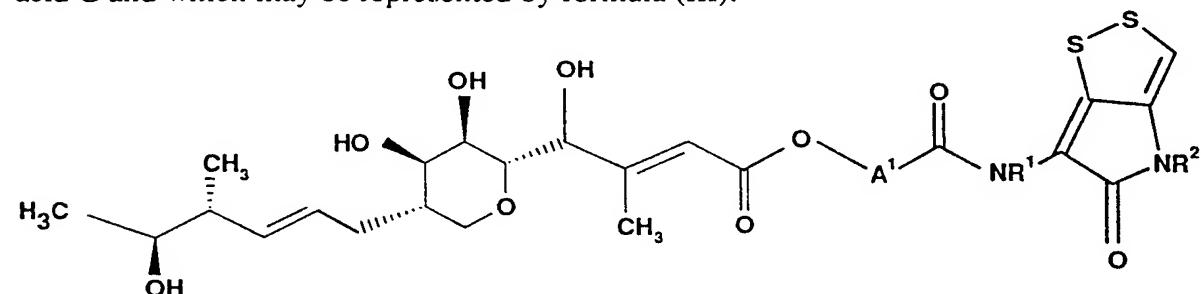
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Accordingly, within the compounds of formula (I), there exists a first sub-set of compounds which are esters of 4-hydroxy monic analogues of acid A and which may be represented by formula (II):



15

and a second sub-set of compounds which are esters of 4-hydroxy analogues of monic acid C and which may be represented by formula (III):



(III)

in which formulae A¹, R¹ and R² are as hereinbefore defined.

- This invention also provides a pharmaceutical or veterinary composition which comprises a compound of formula (I) (hereinafter referred to as the 'drug') together with a pharmaceutically or veterinarily acceptable carrier or excipient. The compositions may be formulated for administration by any route, and would depend on the disease being treated. The compositions may be in the form of, for instance, tablets, capsules, powders, granules, suppositories, lozenges and liquid or gel preparations, including oral, topical and sterile parenteral suspensions.
- Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceutics and cosmetics, such as Harry's Cosmeticology, 7th edn, ed Wilkinson and Moore, 1982, George Godwin, Harlow, England and the British Pharmacopoeia.

Suitable ointment formulations include those described in EP 0 095 897-A (Beecham Group plc), for pseudomonic acid A (mupirocin), and comprise a polyethylene glycol or a polyethylene glycol analogue or derivative, preferably polyethylene glycol 400 optionally admixed with polyethylene glycol 4000.

Suppositories will contain conventional suppository bases, e.g. cocoa-butters

or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the drug and a sterile vehicle. The drug, depending on the vehicle and concentration used, can be suspended in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability the composition can be frozen after filling into the vial and water removed under vacuum. The dry lyophilized powder is then sealed in the vial. The drug can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the drug.

For topical application to the ear, the drug may be made up into a suspension in a suitable liquid carrier, such as water, glycerol, diluted ethanol, propylene glycol, polyethylene glycol or fixed oils. For topical application to the eye, the drug is formulated as a suspension in a suitable, sterile aqueous or non-aqueous vehicle.

15 Additives, for instance buffers such as sodium metabisulphite or disodium edetate; preservatives including bactericidal and fungicidal agents, such as phenylmercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

20 The dosage employed for compositions administered topically will, of course, depend on the size of the area being treated. For the ears and eyes each dose will typically be in the range from 10 to 100 mg of the drug.

Veterinary compositions for intramammary treatment of mammary disorders in animals, especially bovine mastitis, will generally contain a suspension of the drug in an oily vehicle.

25 The compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the drug, depending on the method of administration. Where the compositions are in unit dose form, each dosage unit will preferably contain from 50-500 mg, of the drug. The dosage as employed for adult human treatment (average weight about 70 kg) will preferably range from 100 mg to 3 g per 30 day, for instance 250 mg to 2 g of the drug per day, depending on the route and frequency of administration. Alternatively, the drug may be administered as part of the total dietary intake of a non-human animal. In this case the amount of drug employed may be less than 1% by weight of the diet and in preferably no more than 0.5% by weight. The diet for animals may consist of normal foodstuffs to which the 35 drug may be added or the drug may be included in a premix for admixture with the foodstuff. A suitable method of administration of the drug to animals is to add it to the non-human animal's drinking water. In this case a concentration of the drug in the drinking water of about 5-500 mg/ml, for example 5-200 mg/ml, is suitable.

The compounds of this invention are of use in therapy, in particular for treating bacterial, mycoplasma and/or fungal infections in animals, including humans.

The compounds of this invention are active against both Gram negative and Gram positive organisms, including *Bacteroides*, for instance *B. fragilis* BC1,

5 *Haemophilus*, for instance *H. influenzae* Q1; *Moraxella*, for instance *M. catarrhalis* 1502; *Streptococci*, for instance *S. pyogenes* CN10 and *S. pneumoniae* PU7; *Staphylococci*, for instance *S. aureus* Oxford; *Escherichia*, for instance *E. Coli* DC0, *Legionella*, for instance *L. pneumophila*; *Pseudomonas*, for instance *P. aeruginosa* Dalgleish and *Enterobacter*, for instance *Ent. faecalis* I. In addition, compounds of 10 this invention are active against *Staphylococci* organisms such as *S. aureus* and coagulase negative strains of *Staphylococci* such as *S. epidermidis* which are resistant (including multiply-resistant) to other anti-bacterial agents, for instance, β -lactam antibiotics such as, for example, methicillin; macrolides; aminoglycosides, and lincosamides. Compounds of the present invention are therefore useful in the 15 treatment of MRSA, MRCNS and MRSE. Furthermore, compounds of the present invention are useful in the treatment of *Staphylococci* organisms which are resistant to mupirocin. Bacterial infections which may be treated include Respiratory tract infections, otitis, meningitis, skin and soft tissue infections in man, mastitis in cattle, and respiratory infections in animals such as pigs and cattle. Accordingly, in a further 20 aspect, the present invention provides a method of treating bacterial infection in human or non-human animals, which method comprises administering a therapeutically effective amount of a compound of formula (I) as hereinbefore defined, to a human or non-human animal in need of such therapy.

The compounds of this invention are also active against mycoplasma-induced infections in humans and animals, for instance those caused by *Mycoplasma pneumonia* (human, primary atypical pneumonia), *Mycoplasma gallisepticum* (avian, chronic respiratory diseases), *Mycoplasma bovis* (cattle, mastitis, respiratory diseases and arthritis), *Mycoplasma dispar* (calf, pneumonia), *Mycoplasma hypopneumoniae* (pigs, enzootic pneumonia), *Mycoplasma hyorhinis* (pigs, arthritis) and *Mycoplasma hyposinoviae* (pigs, arthritis). Accordingly in a further aspect, the present invention provides a method of treating mycoplasmal infection in human or non-human animals, which method comprises administering a therapeutically effective amount of a compound of formula (I) as hereinbefore defined, to a human or non-human animal in need of such therapy.

35 In addition, compounds of the present invention are of use in treating infections caused by *Mycoplasma fermentans*, which has been implicated as a co-factor in the pathogenesis of AIDS. Accordingly in a further aspect, the present invention provides a method of treating humans infected with *M. fermentans*, in

particular humans also infected with HIV, which method comprises treating humans in need of such therapy with an anti-mycoplasmal effective amount of a compound of formula (I). It will be appreciated that this method of treatment includes not only the novel compounds of formula (I) but also thiomarinol.

5 The compounds of this invention also have antifungal activity. They may, for example, be used in treating fungal infections in man caused by, among other organisms, species of *Trichophyton*, *Trichosporon*, *Hendersonula*, *Microsporum*, *Epidermophyton*, *Candida*, *Cryptococcus*, *Saccharomyces*, *Paecilomyces* and *Pityrosporum*. They may also be used in the treatment of a variety of other fungal 10 infections caused by, for example *Aspergillus*, *Coccidioides*, *Paracoccidioides*, *Histoplasma* and *Blastomyces* species. Accordingly, in a further aspect, the present invention provides for a method of treating fungal infections in animals, including man, which method comprises treating a patient in need of antifungal therapy with an effect amount of a compound of formula (I). It will be appreciated that this method 15 of treatment includes not only the novel compounds of formula (I) but also thiomarinol.

No adverse toxicological effects are expected from the administration of a compound of formula (I)

Compounds of the present invention, as well as thiomarinol, are also useful 20 as herbicides and are active against a broad range of weed species, including monocotyledonous and dicotyledonous species. Many compounds show good selectivity in crops, particularly wheat, barley, maize, oil seed rape, sugar beet and rice. Compounds for use in herbicidal compositions of the present invention are preferably applied directly to unwanted plants (post-emergence application) but may 25 also be applied to the soil before the unwanted plants emerge (pre-emergence application). Therefore, in a further aspect, the present invention provides for a process of severely damaging or killing unwanted plants which process comprises applying to the plants or the growth medium of the plants a herbicidally effective amount of a compound of formula (I) or thiomarinol.

30 For herbicidal use, compounds of the present invention are preferably used in the form of a composition further comprising a carrier which may be a liquid or solid diluent. Suitable such compositions may be dilute compositions which are ready for immediate use or concentrated compositions which are diluted prior to use, usually with water. Suitable liquid compositions may comprise a solution or a dispersion of 35 the active ingredient in water, optionally with a surfactant, or may comprise a solution or a dispersion of the active ingredient in a water-immiscible organic solvent which is dispersed as droplets in water. Suitable solid compositions may be in the form of granules or dusting powders or dispersible powders or grains, further

comprising a wetting agent to facilitate dispersion. Suitable herbicidal formulating agents are well known in the art; see, for instance, WO 93/19599 (Zeneca Ltd).

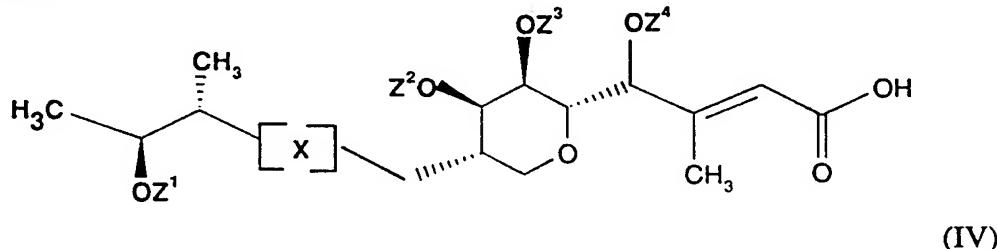
A suitable rate of application for herbicidal use will depend upon the particular application but will usually be in the range 0.0001 to 20kg/hectare,

5 preferably 0.001 to 10kg/hectare, more preferably 0.001 to 2kg/hectare.

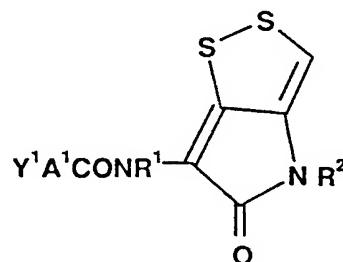
Compounds of the present invention or thiomarinol may be used alone or in admixture with other another herbicide which will preferably have a complementary herbicidal activity in the particular application. Suitable such complementary herbicides are disclosed in WO 93/19599 (Zeneca Ltd)

10 Compounds of formula (I) and thiomarinol may be readily prepared by adapting procedures well known in the art. It will be readily appreciated that compounds of formula (I) are esters of either monic acid A or monic acid C and are therefore obtainable by adapting procedures previously described for other esters of monic acid A in GB 1 587 059 (Beecham Group Ltd). Suitable such procedures
15 include conventional esterification procedures using optionally protected monic acid A or C or an activated derivative thereof and an appropriate "alcohol" comprising a terminal pyrrothin moiety.

Accordingly, in a further aspect, the present invention provides a process for preparing a compound of formula (I) which process comprises esterifying an acid of
20 formula (IV):



in which X is as hereinbefore defined and Z¹, Z², Z³ and Z⁴, which may be the same
25 or different, is each hydrogen or a hydroxyl protecting group;
or a salt or an activated derivative thereof;
with a compound of formula (V):



in which:

Y^1 is a reactive esterifying leaving group; and

A^1 , R^1 and R^2 are as hereinbefore defined;

under ester forming conditions; and

5 thereafter, and if necessary, removing any hydroxyl protecting groups.

Suitably, Y^1 is hydroxy, halogen, preferably bromine or iodine, or sulphonate, preferably halogen or sulphonate.

Suitable ester forming conditions are well known in the art and are described in, for instance, *Comprehensive Organic Synthesis*, Pergamon Press, 1991, 6, 323-

10 380. Suitable ester forming conditions include:

(a) reacting a salt of the acid of the formula (IV), for instance, a sodium or a tertiary amine salt such as triethylamine, with a compound of the formula (V), in a polar aprotic solvent such as dimethyl formamide, dimethyl sulphoxide or acetonitrile, at moderate temperature, for instance in the range 0 to 100°C;

15 (b) reacting the acid of formula (IV) with a compound of formula (V) in the presence of a base such as an alkali metal carbonate or a tertiary amine, in a polar aprotic solvent and temperature as for (a);

(c) reacting the acid of formula (IV) with a compound of formula (V) in which Y^1 is hydroxyl, under dehydrating conditions, for instance the Mitsunobu reaction

20 employing an azodicarboxylate and a trivalent phosphorus reagent (Mitsunobu, *Synthesis*, 1981, 1); or

(d) reacting an activated derivative of the acid of formula (IV), for instance a mixed anhydride, for instance an *iso*-butylcarbonic or a methane sulphonic anhydride, with a compound of formula (V) in which Y^1 is hydroxyl, in the presence of a suitable base such as a tertiary amine, for instance, triethylamine, in an aprotic solvent such as tetrahydrofuran, at a moderate temperature, preferably in the range -20 to +20°C, or alternatively, in the absence of a base but using a preformed salt of the alcohol, for instance the magnesium or lithium alkoxide.

25 Preferred conditions include the use of the sodium salt of the hydroxyl-protected derivative of the acid of formula (IV) in combination with the halide or the sulphonate derivative of the compound of formula (V).

Compounds of formula (IV) (4-hydroxymonic acids A and C and hydroxyl-protected derivatives thereof) and their (C_{1-6})-alkyl esters are novel compounds which are useful as intermediates in the preparation of compounds of formula (I).

30 Accordingly, in a further aspect, the present invention provides for a compound of formula (IV) as hereinbefore defined.

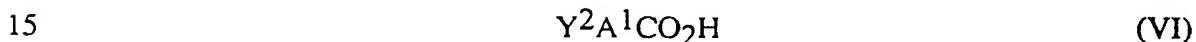
Compounds of formulae (IV) (4-hydroxy monic acids A and C and hydroxyl-protected derivatives thereof) and (VIII) may be obtained in a variety of

ways.

Careful hydrolysis of thiomarinol, obtained by fermentation of the organism *Alteromonas rava* (EP 0 512 824-A, Sankyo Co Ltd), may be used to obtain 4-hydroxymonic acid C. Suitably hydrolysis may be effected using enzymatic processes or whole cell processes. 4-Hydroxymonic acid C may then be converted to the corresponding 4-hydroxymonic acid A by a suitable epoxidation procedure, using for instance m-chlorobenzoic acid in a solvent such as dichloromethane.

Compounds of formula (V) are novel and useful as intermediates in the preparation of compounds of formula (I). Accordingly, in a further aspect, the present invention provides a compound of formula (V), as hereinbefore defined.

Compounds of formula (V) comprise an amide bond and are therefore readily obtainable by conventional amide forming reactions using appropriate acid and amine precursors. A suitable such process comprises reacting an acid of formula (VI):

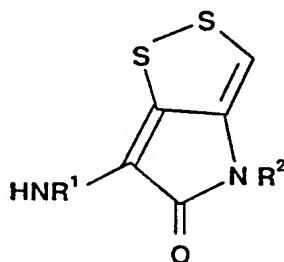


in which:

Y^2 is a protected hydroxyl group or Y^1 (other than hydroxy); and

A^1 is as hereinbefore defined;

with an amine of formula (VII):



20

(VII)

in which R^1 and R^2 are as hereinbefore defined;

under amide forming conditions, as described in, for instance, *Comprehensive*

Organic Synthesis, Pergamon Press, 1991, 6, 381-417, and thereafter, and if

25 necessary, removing any hydroxyl protecting groups;.

Particularly suitable amide forming conditions include reacting an activated derivative of an acid of formula (VI), for instance an acyl halide or a mixed anhydride such as a *iso*-butylcarbonic or methane sulphonic anhydride, with an amine of the formula (VII) in the presence of a suitable base such as a tertiary amine, for instance pyridine, 2, 6-lutidine, triethylamine or 4-dimethylaminopyridine, in an aprotic solvent such as chloroform, dichloromethane or tetrahydrofuran, at a moderate temperature, preferably in the range -30 to +30°C.

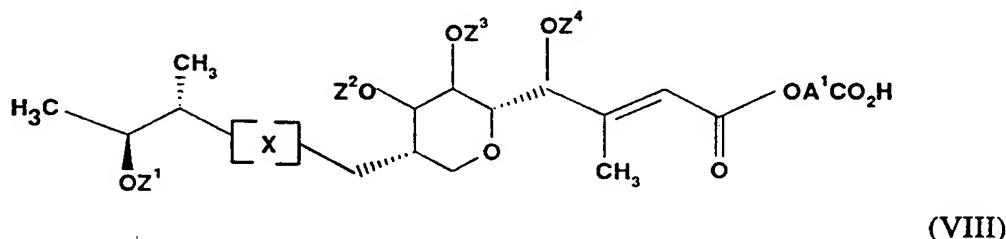
Compounds of formula (VI) may be obtained directly from commercial

suppliers or by conventional modification of compounds which are available from such sources. An amine of formula (VII) may be obtained according to the processes described in GB 2 170 498 A (Imperial Chemical Industries plc) or by semi-synthetic processes starting from natural sources such as thiolutin and holomycin.

- 5 In a second process, compounds of formula (I) may be prepared by a sequence in which the final step comprises forming an amide bond between appropriate acid and amine precursors.

Accordingly, in a further process, a compound of formula (I) may be prepared by a process which comprises reacting an acid of formula (VIII):

10



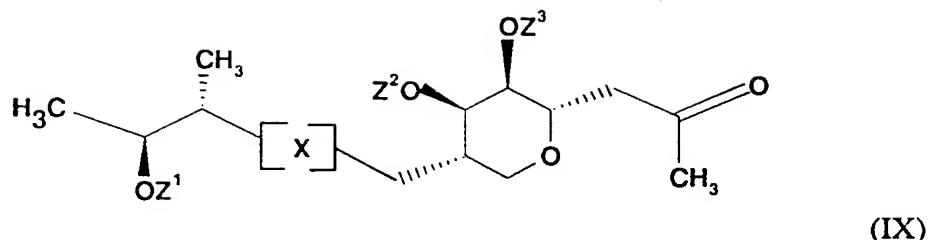
- in which A¹, X, Z¹, Z², Z³ and Z⁴ are as hereinbefore defined; or an activated derivative thereof;
- 15 with an amine of formula (VII) under amide forming conditions; and thereafter, and if necessary, removing any hydroxyl protecting groups.

Suitable amide forming conditions are well known in the art, as hereinbefore described.

- 20 Compounds of formula (VIII) are useful as intermediates in the preparation of compounds of formula (I). Accordingly, in a further aspect, the present invention provides a compound of formula (VIII) as hereinbefore defined and the (C₁₋₆)alkyl esters thereof. Preferred compounds of formula (VIII) include those in which A¹ is (CH₂)_n wherein n is 6, 7 or 8, and salts thereof.

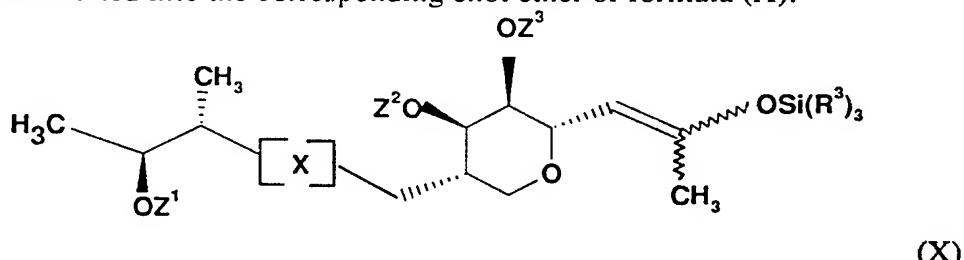
- 25 An acid of formula (VIII) may be obtained by treating a compound of formula (IV) with a compound of formula (VI) in which the carboxyl group is protected by a carboxy protecting group, under esterifying conditions, as hereinbefore described. Suitable carboxy protecting groups are described in Protective Groups in Organic Synthesis, T W Greene and P G M Wuts, Wiley-Interscience, New York, 2nd ed, 1991 and include lower alkyl, preferably methyl, allyl and tetrahydropyranyl.

- 30 4-Hydroxyacids of formula (IV) may be obtained in a variety of ways. In a first process, a ketone of the formula (IX)



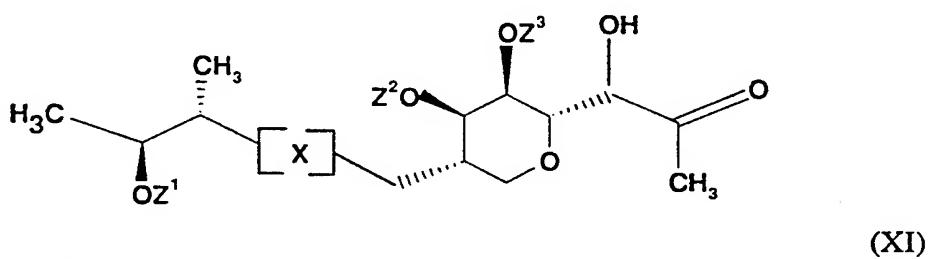
in which X, Z¹, Z² and Z³ are as hereinbefore defined;

is initially converted into the corresponding enol ether of formula (X):



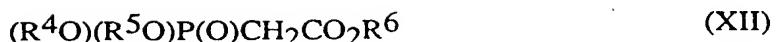
in which R³ is lower alkyl, for instance methyl or *iso*-propyl, preferably *iso*-propyl, and X, Z¹, Z² and Z³ are as hereinbefore defined;

for instance, by treatment thereof with an enolising agent, for instance a strong
 10 organic non-nucleophilic base such as a lithium dialkylamide, for example, lithium
 di-*iso*-propylamide, followed by trapping the enolate thus formed *in situ* with a
 suitable reagent such as tri-*iso*-propylsilyl triflate. The enol ether (X) may then be
 reacted with a reagent capable of introducing an α -hydroxy group, for instance
 15 dimethyldioxirane, by analogy with the procedure described by Adams *et al*, Chem
 Ber, 1991, **124**, 2361 and references therein, to give a α -hydroxy ketone of formula
 (XI):



in which X, Z¹, Z² and Z³ are as hereinbefore defined.

20 This α -hydroxyketone may then be converted into an ester derivative of an
 acid of formula (IV) by initial protection of the α -hydroxy group as, for instance, the
 trimethylsilylether, using for instance, trimethylsilyl chloride and triethylamine,
 followed by an olefination procedure such as those previously described for
 analogues lacking the α -hydroxy substituent in GB 1 587 058 (Beecham Group Ltd).
 25 Suitable such procedures include the following:
 (a) using a metallic derivative, for instance a sodium derivative, of a compound of
 the formula (XII):



in which R^4 and R^5 , which may be the same or different, is each (C_{1-6})alkyl, aryl, or aryl (C_{1-4})alkyl, preferably methyl or ethyl, and R^6 is (C_{1-6})alkyl in a Wadsworth-Emmons reaction;

- 5 (b) using a phosphorus reagent of the formula (XIII):



in which R^7 , R^8 , R^9 , which may be the same or different, is each selected from (C_{1-6})alkyl, aryl or aryl (C_{1-4})alkyl, preferably phenyl, and R^6 is as hereinbefore defined; instead of the reagent derived from the compound of formula (XII) in a

- 10 Wittig reaction; and

(c) using a metallic derivative, for instance a lithium derivative, of a compound of formula (XIV):



in which R^{10} is tri(C_{1-6})alkylsilyl, for instance trimethylsilyl and R^6 is as

- 15 hereinbefore defined, in a Peterson reaction.

The ester derivative thus obtained may be converted into an acid of formula (IV) by suitable hydrolysis, for instance using mild base conditions such as sodium hydroxide in aqueous methanol, or a hydrolase enzyme, such as Subtilisin Carlsberg Protease.

- 20 The α -hydroxyketone of formula (XI) may also be converted into a (C_{1-6})alkyl ester derivative of an acid of formula (VIII) by reaction thereof with a reagent of formula (XII), (XIII) or (XIV) in which R^6 is $A^1CO_2(C_{1-6})alkyl$, in particular $(CH_2)_nCO_2(C_{1-6})alkyl$, wherein n is an integer from 1 to 10. The acid may then be obtained by suitable hydrolysis, as previously described for (IV) (ester \rightarrow acid).

The α -hydroxyketones of formula (XI) are useful intermediates in the preparation of compounds of formula (I). Accordingly, in a further aspect, the present invention provides for a compound of formula (XI) as hereinbefore defined.

A ketone of formula (IX) in which X is an epoxy moiety:

30

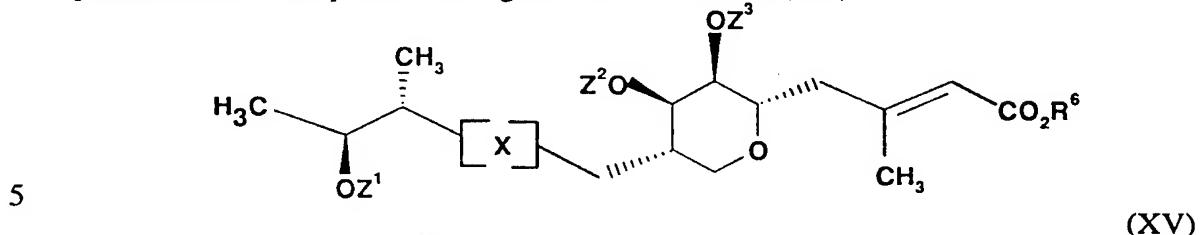


ie, a ketone of the "A" series, is readily obtainable from methyl pseudomonate A, by the ozonolysis thereof, according to the procedure described in GB 1 587 059 (Beecham Group). The corresponding ketone in the "C" series may be readily

- 35 obtained from the corresponding "A" series ketone by a variety of methods, including treatment with sodium iodide/trifluoroacetic anhydride, potassium selenocyanate (Clayton *et al*, J Chem Soc Perkin Trans I, 1981, 287), or a low valent tungsten halide

derivative (Kozlakavski *et al*, J Amer Chem. Soc, 1980, **102**, 6580 and Sharpless *et al*, J. Am Chem Soc, 1992, **94**, 6538).

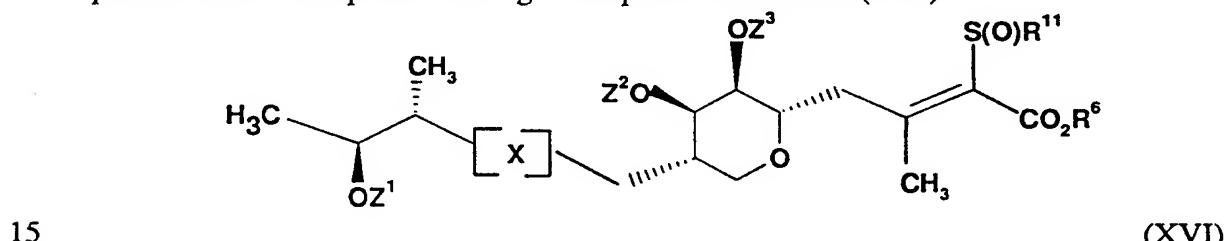
In a second process, 4-hydroxy acids of formula (IV) may be obtained by a process which comprises treating an ester of formula (XV):



in which R⁶, X, Z¹, Z² and Z³ are as hereinbefore defined;

10 with a suitable oxidising agent, for instance selenium dioxide (see March, Advanced Organic Chemistry, reactions, Mechanisms and Structure, McGraw Hill, 3rd edition, 627 and references therein). The preparation of esters of formula (XV) is described in GB 1 587 059.

In a third process, 4-hydroxy acids of formula (IV) may be obtained by a process which comprises treating a compound of formula (XVI):

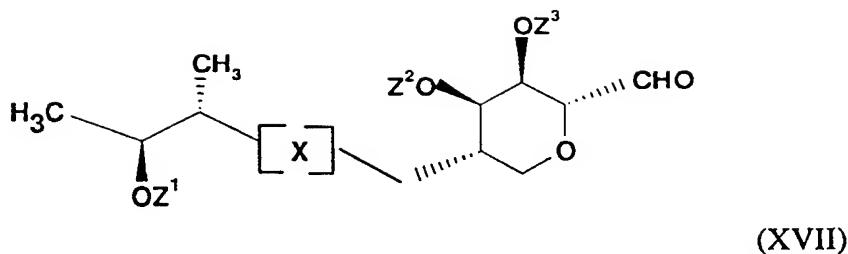


in which R¹¹ is aryl, preferably phenyl, and R⁶, X, Z¹, Z² and Z³ are as hereinbefore defined;

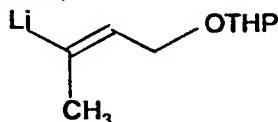
20 with a reagent capable of effecting deconjugation of an α,β -unsaturated ester, for instance N-methylmorpholine, resulting in the introduction of a γ oxygen function *via* a 2,3-sigmatropic rearrangement (see Comprehensive Organic Chemistry, 6, chapter 4.6 and references therein).

Compounds of the formula (XVI) may be obtained by oxidation using, for instance, m-chloroperbenzoic acid in dichloromethane, of the corresponding thioether, itself obtainable by treating the corresponding ester of monic acid A or C with an appropriate non-nucleophilic base, such as lithium di-isopropylamide, followed by a sulphur electrophile such as diphenyl disulphide (to give R¹¹ as phenyl) (see Crimmin *et al*, J Chem Soc Perkin Trans I, 1985, 549).

Acids of formula (IV) (in the "A" series) may also be obtained by a process which comprises treating an aldehyde of formula (XVII):

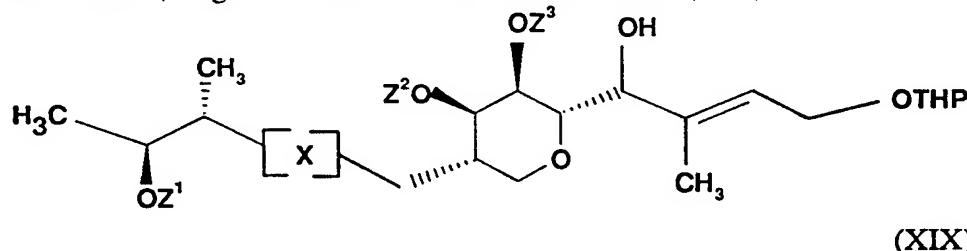


in which X, Z¹, Z² and Z³ are as hereinbefore defined;
with a reagent of the formula (XVIII):



5

in which THP denotes the tetrahydropyranyl protecting group, or a functional equivalent thereof; to give an intermediate of the formula (XIX):



10

in which X, Z¹, Z² and Z³ are as hereinbefore defined;
which may then, after the 4-hydroxyl group has been protected and the THP protecting group removed, be converted to an acid of formula (IV) using, for instance, manganese dioxide, by analogy with the procedure described by Williams *et al*, J. Org. Chem., 1986, 51, 3916. For the "A" series, an aldehyde of formula (XVII) may be obtained by ozonolysis of the enol ether of formula (X). For the "C" series, a suitable intermediate of formula (XIX) is described by Williams *et al* (*ibid*).

15

It will be readily appreciated by the skilled man that the various methods hereinbefore described for introducing the hydroxy substituent, to provide appropriate precursors for further elaboration into compounds of formula (I), in general lack stereospecificity and will therefore lead to the formation of intermediates in which the hydroxyl group has both possible configurations. Such diastereoisomers may be readily separated at a convenient stage in the overall synthesis, by conventional methods used therefor, such as chromatography or fractional crystallisation. This process may be assisted if the hydroxyl group is in a protected form.

20

Monic acid A may be readily obtained from pseudomonic acid A by the carefully controlled hydrolysis thereof, according to the process described in GB 1 587 058 (Beecham Group Ltd). A similar process may be used to obtain monic acid C from pseudomonic acid C (Clayton J P *et al*, JCS Perkin Trans I, 1982, 2827).

Alternatively, monic acid C may be obtained from monic acid A by the deoxygenation thereof, according to the process described in EP 0 003 069 (Beecham Group Ltd).

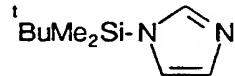
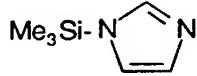
It will be readily appreciated that compounds of formula (II) and (III) of monic acid A and monic acid C and hydroxyl-protected derivatives thereof, are readily interconvertible by suitable deoxygenation and epoxidation procedures, for instance by analogy with those described for monic acids A and C in EP 0 003 069-A (Beecham Group plc) and by Clayton J P *et al*, JCS Perkin Trans I, 1982, 2827.

It will be appreciated that the various processes described herein also may be used to prepare the natural product thiomarinol which has previously only been available as a fermentation product. Accordingly, in a further aspect, the present invention provides processes for the preparation of thiomarinol, which processes are those hereinbefore described for compounds of formula (I).

When used herein, the term 'hydroxyl-protecting group' refers to any such group known in the art which may be removed without disruption of the remainder of the molecule. Suitable hydroxyl-protecting groups are described in Protective Groups in Organic Synthesis, T.W. Greene, Wiley-Interscience, New York, 2nd ed, 1991.

The hydroxyl groups of the compound of formulae (IV), (VIII), (IX), (X), (XI), (XV), (XVI), (XVII) and (XIX) may be protected at any stage of the above processes, using conventional methods. The hydroxyl-protecting group may be removed by methods known in the art, including enzymatic methods. Particularly suitable hydroxyl-protecting groups are silyl groups since these are readily removed under mild conditions. Such groups are introduced using conventional silylating agents, including halosilanes and silazanes, for example those of the following formulae :

L_3SiY ; L_2SiY_2 ; L_3SiNL_2 ; $L_3SiNHSiL_3$; $L_3SiNHCOL$; $L_3SiO-C(L)=NSiL_3$;
 $L_3SiNHCONHSiL_3$; $LNHCONHSiL_3$; $tBuMe_2Si-O-SO_2-CF_3$;



in which Me denotes methyl, t-Bu denotes t-butyl, Y is halogen and each group L is independently selected from hydrogen, (C₁₋₆)alkyl, (C₁₋₆)alkoxy, aryl or aryl(C₁₋₄)alkyl. A preferred silylating agent is trimethylsilyl chloride. Particularly suitable hydroxyl-protecting groups are trimethylsilyl, triethylsilyl and t-butyldimethylsilyl groups. Preferred hydroxyl-protecting groups are trimethylsilyl groups because of their ease of removal.

The glycol function of the compound of formulae (IV), (VIII), (IX), (X), (XI), (XV), (XVI), (XVII) and (XIX) may be protected by forming a cyclic derivative using a compound of formula (XX):



in which R^a is hydrogen or (C_{1-6})alkyl and each of R^b , R^c and R^d is (C_{1-6})alkyl such that in the cyclic derivative Z^2 and Z^3 together are a moiety $R^bC(OR^c)$.

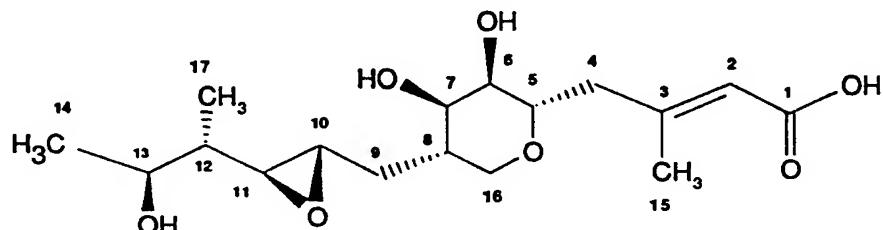
Suitably R^a is hydrogen, methyl, ethyl, *n*- or *iso*-propyl; most suitably it is hydrogen.

- 5 The groups R^b , R^c and R^d are suitably methyl, ethyl, *n*- or *iso*-propyl, or *n*-, *iso*-, *sec*- or *t*-butyl; most suitably methyl. The hydroxyl groups of a compound of formula (I) may also be protected prior to conversion to a further compound of formula (I) as described above. In each case the protecting groups described above may be removed by mild acid hydrolysis followed by alkaline hydrolysis, for instance, as described by
- 10 Clayton J P *et al*, JCS Perkin Trans I, 1979, 308.

The following examples illustrate the invention, but are not intended to limit the scope in any way:

General - It is to be noted that in the following preparations and examples, in the assignments given in the proton and carbon nmr spectral data, the numbering system used is based upon that of monic acid, following the normal convention in natural

- 15 product chemistry, rather a systematic numbering for that particular compound. This numbering system is shown below:



20 Preparations

Preparation 1 - Methyl 4-hydroxymononate A

- a) **Methyl 2-phenylthiomononate A** - *n*-Butyllithium (12.34ml of a 1.6M solution in hexane) was added to a stirred solution of diisopropylamine (2.77ml) in dry tetrahydrofuran (THF) (20ml) at -30°C under dry argon. After 40 minutes at -30°C the mixture was cooled to -78°C and treated, dropwise over 10 minutes, with a solution of methyl tristrimethylsilylmononate A (9.19g) in dry THF (40ml). After 1 hour at -78°C the stirred mixture was treated, dropwise over 5 minutes, with a solution of diphenyldisulphide (4.30g) in dry THF (20ml). The mixture was allowed to attain room temperature and stirred for 18 hours. The mixture was treated with saturated aqueous ammonium chloride solution (30ml) and extracted with ethyl acetate. The organic layer was washed with water and evaporated. The residue was redissolved in THF (420ml) and treated with hydrochloric acid (84ml of a 0.4M aqueous solution). After 2 minutes the stirred mixture was treated with saturated

sodium bicarbonate solution (84ml). The mixture was evaporated to low volume and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated. The residual oil was chromatographed on silica gel eluting with methanol/dichloromethane mixtures to give the title compound as 1:1 mixture of *E* and *Z* isomers (5.9g); δ_H (CD_3OD) 0.94 (1.5H, d, *J* 7.1Hz, 17- H_3), 0.95 (1.5H, d, *J* 7.0Hz, 17- H_3), 1.20 (1.5H, d, *J* 6.3Hz, 14- H_3), 1.20 (1H, d, *J* 6.4Hz, 14- H_3), 1.42 (1H, m, 12-H), 1.68 (2H, m, 9- H_2), 1.96 (1H, m, 8-H), 2.10 (1.5H, s, 15- H_3), 2.14 (1.5H, s, 15- H_3), 2.65-2.72 (1H, m, 10-H), 2.72-2.88 (3H, m, 4- H_2 and 11-H), 3.44 (1H, m, 6-H), 3.53 (1.5H, s, CO_2CH_3), 3.56 (1.5H, s, CO_2Me), 3.6-3.9 (5H, m, 5-H, 6-H, 7-H, 13-H and 16-H) and 7.1-7.4 (5H, m, Ar-H); m/z (NH_3^+DCI) 467 (28%, MH^+) and 484 (100%, MNH_4^+).

b) **Methyl 2-phenylthio (6,7,13-O-tristrimethylsilyl)monate A** - The mixture of isomers from Preparation 1a (5.9g) was dissolved in dry THF (150ml) and treated with triethylamine (5.05ml), 4-dimethylamino-pyridine (50mg), and chlorotrimethylsilane (4.6ml). After 2 hours at room temperature the mixture was treated with triethylamine (2.6ml) and chlorotrimethylsilane (2.3ml). After an additional 2.5 hours at room temperature the mixture was filtered and evaporated to give a mixture of *Z* and *E* isomers of the title compound. Chromatography of this mixture on silica gel eluting with ethyl acetate/hexane mixtures afforded the less polar (*Z*)-isomer of the title compound (2.13g); ν_{max} (KBr) 2955, 1718, 1250, 1124, and 841 cm^{-1} ; λ_{max} (EtOH) 201.5nm (ϵ_m 20165) and 250.5 (ϵ_{max} 10976)nm; δ_H ($CDCl_3$) 0.10, 0.13 and 0.19 (27H, each s, 6,7,13-OSi(CH_3)₃), 0.9 (3H, d, *J* 7.0Hz, 17- H_3), 1.20 (3H, d, *J* 6.3Hz, 14- H_3), 1.38 (1H, m, 12-H), 1.57 (1H, m, 9-H), 1.75 (1H, m, 9-H), 1.80 (1H, m, 8-H), 2.15 (3H, s, 15- H_3), 2.58-2.70(3H, m, 4-H, 10-H and 11-H), 2.76 (1H, dd, *J* 2.4 and 13.8Hz, 4-H), 3.37 (1H, dd, *J* 2.4 and 8.5Hz, 6-H), 3.51 (1H, d, *J* 11.3Hz, 16-H), 3.57 (3H, s, CO_2CH_3), 3.78 (2H, m, 5-H and 7-H), 3.86 (2H, m, 13-H, and 16-H), 7.12 (1H, m, ArH), and 7.26 (4H, m, ArH); δ_C ($CDCl_3$) 0.22, 0.39, and 0.54 (Si-C), 12.47 (C-17), 20.87 (C-15), 22.06 (C-14), 32.17 (C-9), 38.70 (C-4), 41.92 (C-8), 42.74 (C-12), 51.66 (OCH₃), 55.33 (C-10), 59.34 (C-11), 65.38 (C-16), 70.41 (C-6), 71.20 (C-7), 73.15 (C-13), 75.36 (C-5), 122.62 (C-2), 125.70, 127.90, 128.79 and 136.0 (aromatics), 154.65 (C-3), and 167.58 (C-1); m/z (NH_3^+DCI) 683 (20%, MH^+) and 700 (100%, MNH_4^+). Found: M^+ 682.3222. $C_{33}H_{58}O_7SSi_3$ requires m/z 682.3211.

c) **Methyl (Z)-(2-phenylsulphonyl (6,7,13-O-tristrimethylsilyl) monate A** - A solution containing *m*-chloroperbenzoic acid (126mg) in chloroform (2ml) was added portionwise to a stirred solution of the product from Preparation 1b (500mg) in chloroform (2.5ml). After the reaction had completed (tlc monitoring) the mixture was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated. The

residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give the title compound as a 1:1 mixture of *R* and *S* sulphoxides (489mg); δ_H (CD₃OD) 0.92 (1.5H, d, *J* 6.9Hz, 17-H₃), 0.92 (1.5H, d, *J* 7.0Hz, 17-H₃), 1.19 (3H, d, *J* 6.3Hz, 14-H₃), 1.35 (1H, m, 12-H), 1.67 (2H m, 9-H₂), 1.90 (1H, m, 8-H), 2.37 (1.5H, s, 15-H₃), 2.40 (1.5H, s, 15-H₃), 2.50 (1H, m, 4-H), 2.73 (2H, m, 10-H and 11-H), 2.82 (1H, m, 4-H), 3.4 (1/2H, d, *J* 2.4Hz, 1/2 6-H), 3.45 (2H, s, CO₂CH₃ and 1/2 6-H), 3.50 (1.5H, s, CO₂CH₃), 3.55 (1H, m, 16-H), 3.71 (1H, m, 5-H), 3.78-3.95 (3H, m, 7-H, 13-H and 16-H) and 7.5-7.6 (5H, m, Ar-H). *m/z* FAB (3-NOBA) 721 (MNa⁺)

5 d) **Methyl 4-hydroxy (6,7,13-O-tristrimethylsilyl)monate A** - A mixture of the product from Preparation 1c (665mg), N-methylmorpholine (1.5ml), and acetonitrile/water (3:1, 10ml) was stirred under argon for 6.5 hours. The mixture was diluted with ethyl acetate and washed with water. The aqueous layer was extracted with ethyl acetate. The combined organic layers were dried (MgSO₄), evaporated, and the residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give an approximately 2:1 mixture of diastereomers of the title compound (63mg); δ_H (CD₃OD) 0.91 (1H, d, *J* 7.0Hz, 17-H₃), 0.93 (2H, d, *J* 7.0Hz, 17-H₃), 1.19 (1H, d, *J* 6.3Hz, 14-H₃), 1.20 (2H, d, *J* 6.3Hz, 14-H₃), 1.25-1.45 (2H, m, 9-H and 12-H), 1.6-1.7 (2H, m, 9-H and 8-H), 2.11 (2H, d, *J* 0.7Hz, 15-H₃), 2.13 (1H, d, *J* 1.1Hz, 15-H₃), 2.75 (2H, m, 10-H and 11-H), 3.48 (0.33H, d, *J* 11.23Hz, 16-H), 3.55 (0.66, d, *J* 11.23Hz, 16-H), 3.67 (3H, s, CO₂CH₃), 3.73 (1H, dd, *J* 1.4 and 9.1Hz, 6-H), 3.9-4.03 (3H, m, 5-H, 7-H and 13-H), 4.15 5.98 (0.33H, br.s, 4-H), 5.98 (0.33H, br. s, 2-H) and 6.02 (0.66H, t, *J* 1.3Hz, 2-H); *m/z* (NH₃⁺DCI) 591 (20%, MH⁺) and 608 (25%, MNH₄⁺).

10 e) **Methyl 4-hydroxymonate A** - The product from Preparation 1d (146mg) was dissolved in THF (6ml) and treated with hydrochloric acid (1.2ml of 0.4M solution). After 2 minutes the stirred mixture was treated with saturated sodium bicarbonate (1.2ml). The mixture was diluted ethyl acetate and the organic layer separated, washed with brine, dried (MgSO₄), and evaporated. Chromatography of the residue on silica gel eluting with methanol/ dichloromethane mixtures gave two fractions. The first fraction contained the less polar isomer of the title compound (19mg); δ_H (CDCl₃) 0.92 (3H, d, *J* 7.1Hz, 17-H₃), 1.21 (3H, d, *J* 6.3Hz, 14-H₃), 1.34 (1H, m, 12-H), 1.72 (2H, m, 9-H₂), 2.01 (1H, m, 8-H), 2.17 (3H, d, *J* 1.2Hz, 15-H₃), 2.52 (1H, br. s, OH, D₂O exch.), 2.70 (1H, dd, *J* 2.2 and 8.0Hz, 11-H), 2.78 (1H, dt, *J* 2.2 and 6.3Hz, 10-H), 2.97 (1H, br. s, OH, D₂O exch.), 3.31 (1H, br. s, OH, D₂O exch.), 3.54 (1H, br. s, OH, D₂O exch.), 3.60 (1H, dt, *J* 1.1 and 12Hz, 16-H), 3.64 (1H, dd, *J* 6.4 and 9.3Hz, 5-H), 3.70 (3H, s, CO₂CH₃), 3.78 (1H, dd, *J* 3.3 and 9.3Hz, 6-H), 3.79 (1H, dq, *J* 6.3 and 6.4Hz, 13-H), 3.89 (1H, dd, *J* 2.75 and 11.6Hz, 16-H), 3.94

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- (1H, ddd, *J* 1.1, 2.8 and 3.2, 7-H), 4.22 (1H, br d collapsing to dd, *J* 6.5 and *c* 1Hz on D₂O exch., 4-H), and 5.97 (1H, m, 2-H); δ_C (CDCl₃) 12.8 (C-17), 15.0 (C-15), 20.8 (C-14), 31.55 (C-9), 38.9 (C-8), 42.7 (C-12), 51.17 (OCH₃), 55.74 (C-10), 61.33 (C-11), 65.62 (C-16), 67.62 (C-6), 70.1 (C-7), 71.5 (C-13), 75.6 (C-5), 78.95 (C-4), 117.21 (C-2), 157.1 (C-3), and 167.1 (C-1); *m/z* (NH₃⁺DCI) 375 (10%, MH⁺), 392 (14%, MNH₄⁺). Found: MH⁺, 375.2017. C₁₈H₃₀O₈ requires *m/z* 375.2019. The second fraction contained the more polar isomer of the title compound (43mg); δ_H (CDCl₃) 0.92 (3H, d, *J* 7.0Hz, 17-H₃), 1.21 (3H, d, *J* 6.3Hz, 14-H₃), 1.34 (1H, m, 12-H), 1.69 (2H, m, 9-H₂), 2.02 (1H, m, 8-H), 2.14 (3H, s, 15-H₃), 2.7-2.82 (3H, m, 10-H, 11-H and 10-H₂), on D₂O exch. collapsed to 2.73 (1H, dd, *J* 2.2 and 7.6Hz, 10-H) and 2.78 (1H, dt, *J* 2 and 5Hz, 11-H), 3.11 (1H, s, OH, D₂O exch.), 3.33, (1H, d, *J* 9.2Hz, D₂O exch.), 3.48 (1H, m, OH, D₂O exch.), 3.61 (1H, d, *J* 13.4Hz, 16-H), 3.67 (1H, dd, *J* 1.7 and 9.7Hz, 5-H), 3.69 (3H, s, CO₂CH₃), 3.78 (1H, m, 13-H), 3.86 (1H, m, 6-H), 3.88 (1H, dd, *J* 2.5 and 11.65Hz, 16-H), 3.99 (1H, s, 7-H), 4.29 (1H, br d, *J* 8.9Hz, 4-H), and 6.02 (1H, br.s, 2-H); δ_C (CDCl₃) 12.8 (C-17), 16.0 (C-15), 21.0 (C-14), 31.5 (C-9), 39.6 (C-8), 42.8 (C-12), 51.0 (OCH₃), 55.6 (C-10), 61.1 (C-11), 64.7 (C-6), 65.6 (C-16), 70.5 (C-7), 71.4 (C-13), 73.7 (C-4), 75.9 (C-5), 115.5 (C-2), 158.5 (C-3), and 167.2 (C-1); *m/z* (NH₃⁺DCI) 375 (15%, MH⁺), 392 (20%, MNH₄⁺). Found: MH⁺, 375.2017. C₁₈H₃₀O₈ requires *m/z* 375.2019.
- 20 Preparation 2: 2R,3R,4R,5S-2-Formyl-3,4-bis-triethylsilyloxy-5-(2S,3S-epoxy-5S-triethylsilyloxy-4S-methylhexyl)tetrahydropyran
- a) 2S,3R,4R,5S-5-(2S,3S-Epoxy-5S-triethylsilyloxy-4S-methylhexyl)-2-(2-oxoprop-1-yl)-3,4-bis-triethylsilyloxytetrahydropyran - To a solution of Pseudomonic acid A (23g, 46mmol) in collidine (65ml) at -15°C was added 25 triethylsilyl trifluoromethane sulphonate (57ml, 255mmol) over a period of 10 minutes. After a further 20 minutes the mixture was treated with saturated aqueous ammonium chloride solution (270ml) followed by diethyl ether (180ml). The reaction mixture was stirred at room temperature for 16 hours. The organic layer was separated and diluted to 400ml with diethyl ether. This was then washed with 10% aqueous citric acid solution, water, and saturated brine, dried over anhydrous 30 magnesium sulphate and evaporated under reduced pressure.
- The oily residue was dissolved in dichloromethane (500ml) and cooled to -70°C. Ozone was bubbled through until a blue colour was observed, then argon was bubbled through to remove excess ozone. The solution was treated with 35 triphenylphosphine (14.46g, 55mmol), the mixture allowed to warm to room temperature and stirred for 2 days. The reaction mixture was evaporated and the residue chromatographed on Kieselgel 60 eluting with 0-20% ethyl acetate in hexane to afford the title product as a colourless oil (20.7g, 70%); δ_H (CDCl₃, *inter alia*)

0.56-0.68 (18H, m, SiCH₂), 0.91-1.02 (30H, m, SiCH₂CH₃ + 17-H₃), 1.19 (3H, d, *J* 6.4Hz, 14-H₃), 2.18 (3H, s, 15-H₃), 2.41 (1H, dd, *J* 10.1, 15.2Hz, 4-H), 2.64-2.73 (3H, m, 10,11, + 4'-H), 3.40 (1H, dd, *J* 2.2, 9.2Hz, 6-H), 4.14 (1H, dt, *J* 3.0, 9.9Hz, 5-H).

- 5 b) **2S,3R,4R,5S-2-(Z-2-tri-isopropylsilyloxyprop-1-enyl)-3,4-bis-triethylsilyloxy-5-(2S,3S-epoxy-5S-triethylsilyloxy-4S-methylhexyl)tetrahydropyran** - The product from Preparation 2a (0.466g, 1.0mmol) was dissolved in dry tetrahydrofuran (4ml) and added to a solution of lithium diisopropyl-amide (0.85mmol) in dry tetrahydrofuran (4ml) at -70°C under an argon atmosphere. After 10 minutes, the solution was allowed to warm to -30°C and stirred at that temperature for 10 minutes. The solution was re-cooled to -70°C, treated with triisopropyltrifluoromethane sulphonate (*ex* Aldrich Chemical Company) (0.268ml, 1.0mmol), warmed to -20°C and stirred for 30 minutes at this temperature. The cooling bath was removed and the solution warmed to room temperature over a period of 30 minutes. The solvent was evaporated under reduced pressure, the residue taken up in dry pentane (10ml) and the mixture filtered. The filtrate was evaporated under reduced pressure and the residue chromatographed on Kieselgel 60 eluting with 0-5% ethyl acetate in hexane to afford the title compound as a colourless oil (122mg, 15%); δ_H (CDCl₃, *inter alia*) 1.21 (3H, d, *J* 6.4Hz, 14-H₃), 1.86 (3H, s, 15-H₃), 2.64-2.71 (2H, m, 10-H and 11-H), 3.41 (1H, dd, *J* 1.8, 9.3Hz, 6-H), 3.50 (1H, d, *J* 11.2Hz, 16-H), 4.39 (1H, d, *J* 9.1Hz, 4-H), 4.60 (1H, appears as t, *J* 9.2Hz, 5-H); *m/z* (+ve ion FAB, 3-NOBA/Na) 823 (MNa⁺, 30%).
- 20 c) **2R,3R,4R,5S-2-Formyl-3,4-bis-triethylsilyloxy-5-(2S,3S-epoxy-5S-triethylsilyloxy-4S-methylhexyl)tetrahydropyran** - The product from Preparation 2b (0.122g, 0.15mmol) was dissolved in dry dichloromethane (10ml), cooled to -70°C, and ozone passed through until the solution was blue in colour. Argon was blown through to remove excess ozone, and the solution treated with dimethylsulphide (0.040ml, 0.55mmol). the cooling bath was removed and the solution allowed to warm to room temperature over a period of 2 hours. The resulting solution was washed with water (5ml), saturated aqueous sodium chloride (5ml), dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The residue was chromatographed on Kieselgel 60 eluting with 10% ethyl acetate in hexane to afford the title compound as a colourless oil (11mg, 12%); δ_H (CDCl₃, *inter alia*) 1.18 (3H, d, *J* 6.4Hz, 14-H₃), 2.64-2.70 (2H, m, 10 + 11-H), 3.35 (1H, d, *J* 6.6Hz, 6-H), 3.50 (1H, d, *J* 11.3Hz, 16-H), 4.25 (1H, dd, *J* 6.6, 1.4Hz, 5-H), 9.8 (1H, s, CHO); *m/z* (NH₃⁺DCI) 617 (40%, MH⁺), 634 (30%, MNH₄⁺).

Preparation 3: Methyl 4-trimethylsilyloxy (6,7,13-O-tristrimethylsilyl)monate A

- a) **2S,3R,4R,5S-2-(Z-2-tri-isopropylsilyloxyprop-1-enyl)-3,4-bis-**

trimethylsilyloxy-5-(2S,3S-epoxy-5S-trimethylsilyloxy-4S-methylhexyl)tetrahydropyran - A solution of 5S-(2S,3S-epoxy-5S-trimethylsilyloxy-4-methylhexyl-3R,4R-bis-trimethylsilyloxytetrahydropyran-2S-yl)acetone [6,7,13-O-tris-trimethylsilylmonone] GB 1 587 058 (6.16g, 12mmol) in dry tetrahydrofuran (20ml)

5 was added to a solution of lithium diisopropyl-amide (10mmol) in dry tetrahydrofuran (20ml) at -70°C under an argon atmosphere. The cooling bath was removed, the mixture warmed to room temperature over a period of 15 minutes, and stirred for a further 45 minutes. Tri-isopropylsilyl trifluoromethane sulphonate (2.55ml, 10mmol) was then added, and the mixture stirred for a further hour. The 10 solvent was evaporated under reduced pressure, the residue taken up in dry pentane (10ml) and the mixture filtered. The filtrate was evaporated under reduced pressure and the residue chromatographed on Kieselgel 60 eluting with 2%-5% ethyl acetate in hexane to afford the title compound as a colourless oil (2.86g, 42%); δ_H (CDCl₃) 0.91 (3H, d, *J* 7.1Hz, 17-H₃), 1.21 (3H, d, *J* 6.4Hz, 14-H₃), 1.56 (3H, s, CH₃), 2.64-15 2.68 (2H, m, 10-H and 11-H), 3.42 (1H, dd, *J* 2.3, 8.3Hz, 6-H), 3.49 (1H, d, *J* 11.4Hz, 16-H), 3.75 (1H, appears as br.s, 7-H), 3.81-3.91 (1H, m, 13-H), 3.97 (1H, d, *J* 11.4Hz, 16'-H), 4.38 (1H, d, *J* 9.3Hz, 4-H), 4.62 (1H, appears as t, *J* 9.1Hz, 5-H); *m/z* 674 (*M*⁺), 117 (100%).

b) **3R,4R-Dihydroxy-5S-(2S,3S-epoxy-5S-hydroxy-4S-methylhexyl)-2R-(1-hydroxy-2-oxoprop-1-yl)tetrahydropyran** - Dimethyldioxirane (J.Org.Chem., 1987, 52, 2800) (15ml, of a 0.08M solution in acetone) was added to a stirred solution of the product from Preparation 3a (0.75g) in dichloromethane (9ml) at -40°C. The temperature of the mixture was allowed to rise to -20°C and stirring was continued for 30 minutes. The temperature was allowed to rise to 0°C during a further 25 15 minutes and the mixture was purged thoroughly with argon (10 minutes). The mixture was evaporated and the residue redissolved in THF (18ml) and treated with hydrochloric acid (3.6ml, of 0.4M). After 2 minutes the stirred mixture was treated with saturated sodium bicarbonate solution (4ml). The mixture was evaporated and the residue was triturated with methanol. The mixture was filtered and the filtrate 30 evaporated and chromatographed on silica gel eluting with methanol/dichloromethane mixture to give a 3:2 mixture of diastereomers of the title compound (343mg); ν_{max} (KBr) 3410 and 1720cm⁻¹; δ_H (CD₃OD) 0.94 (3H, d, *J* 7.0Hz, 17-H₃), 1.20 (3H, d, *J* 6.4Hz, 14-H₃), 1.40 (1H, m, 12-H), 1.60-1.80 (2H, m, 9-H₂), 1.91 (1H, m, 8-H), 2.21 and 2.2 (3H, each s, COCH₃), 2.70 (1H, m, 10-H), 2.79 (1H, m, 11-H), 35 3.54 and 3.61 (1H, each d, *J* 11.3 and 11.45Hz), 3.75 and 3.98 (5H, m, 5-H, 6-H, 7-H, 13-H and 16-H), 4.24 (0.4H, d, *J* 2.3Hz, 4-H) and 4.32 (0.6H, d, *J* 1.7Hz, 4-H); *m/z* (+ve ion FAB, 3-NOBA/Na) 557 (*M*Na⁺); δ_C (CD₃OH) 12.18 (C-17), 27.34 and 27.02 (C-14), 32.61 and 32.72 (C-9), 41.58 and 41.94 (C-8), 43.73 (C-12), 56.84 and

56.92 (C-4), 61.21 (C-10), 65.04 (C-11), 66.38 and 66.56 (C-16), 70.67 (C-6), 71.59 and 71.65 (C-7), 77.46 and 78.28 (C-13), 79.10 and 79.46 (C-5) and, 211.17 and 212.22 (C-3); m/z (NH₃⁺DCI) 319 (45%, MH⁺) and 336 (100%, MNH⁺). Found: MH⁺, 319.1749. C₁₅H₂₆O₇ requires 319.1757).

- 5 c) **3R,4R-Bistrimethylsilyloxy-5S-(2S,3S-epoxy-4S-methyl-5S-trimethylsilyloxyhexyl)-2R-(2-oxo-1-trimethylsilyloxyprop-1-yl)tetrahydropyran** - A mixture of the product of Preparation 3b (269mg), triethylamine (0.55ml), 4-dimethylaminopyridine (35mg), and chlorotrimethylsilane (0.61ml) in dry THF (9ml) was stirred at room temperature for 2 hours. The mixture diluted with ether, 10 filtered, and the filtrate evaporated. Chromatography of the residue on silica gel eluting with ethyl acetate/hexane mixtures gave two fractions. The first fraction contained the less polar isomer of the title compound (121mg); ν_{max} (KBr) 2956, 1730, 1251 and 1132cm⁻¹; δ _H (CDCl₃) 0.02-0.22 (36H, m, SiCH₃), 0.89 (3H, d, *J* 7.1Hz, 17-H₃), 1.18 (3H, d, *J* 6.3Hz, 14-H₃), 1.35-1.46 (1H, m, 12-H), 1.48-1.58 15 (1H, m, 9-H), 1.58-1.7 (1H, m, 9-H), 1.75 (1H, b.s, 8-H), 2.16 (3H, d, *J* 1.7Hz, COCH₃), 2.6-2.7 (2H, m, 10-H and 11-H), 3.5 (1H, dd, *J* 1 and 11Hz, 16-H), 3.79- 3.88 (3H, m, 6-H, 7-H and 13-H), 3.9 (1H, dd, *J* 1.6 and 9Hz, 5-H), 3.93 (1H, dd, *J* 2.6 and 11.4Hz, 16-H) and 4.16 (1H, d, *J* 1.6Hz, 4-H); δ _C (CDCl₃) 12.46 (C-17), 20.86 (C-14), 27.59 (C-15), 31.73 (C-9), 41.71 (C-8), 42.6 (C-12), 55.34 (C-10), 20 59.45 (C-11), 65.57 (C-16), 66.21 (C-6), 70.45 (C-7), 72.99 (C-13), 78.04 (C-5), 79.88 (C-4) and 210.25 (C-3); m/z (NH₃⁺DCI) 607 (10%, MH⁺) and 624 (28%, MNH₄⁺); Found: C, 53.47; H, 9.55%. C₂₇H₅₈O₇Si₄ requires: C, 53.42; H, 9.63%. The second fraction contained the more polar isomer of the title compound (180mg); ν_{max} (KBr) 2957, 1735, 1250, 1090 and 841cm⁻¹; δ _H (CDCl₃) 0.02-0.26 (36H, m, SiCH₃), 0.89 (3H, d, *J* 7Hz, 17-H₃), 1.19 (3H, d, *J* 6.3Hz, 14-H₃), 1.35-1.45 (1H, m, 12-H), 1.53-1.63 (1H, m, 9-H), 1.72-1.89 (2H, m, 9-H and 8-H), 2.16 (3H, s, COCH₃), 2.62-2.72 (2H, m, 10-H and 11-H), 3.50 (1H, d, *J* 11.4Hz, 16-H), 3.81-3.9 (3H, m, 6-H, 13-H and 16-H), 3.93 (1H, t, *J* 3.3Hz, 7-H), 3.97 (1H, dd, *J* 4.5 and 8.8Hz, 5-H) and 4.38 (1H, d, *J* 2.5Hz, 4-H); δ _C (CDCl₃) 12.46 (C-17), 20.95 (C-14), 25 25.93 (C-15), 31.68 (C-9), 41.16 (C-8), 42.75 (C-12), 55.00 (C-10), 59.67 (C-11), 65.18 (C-16), 66.53 (C-6), 70.49 (C-7), 73.08 (C-13), 79.1 (C-5), 77.37 (C-4) and 208.23 (C-3); m/z (NH₃⁺DCI) 607 (5%, MH⁺) and 624 (28%, MNH₄⁺); Found: C, 53.00; H, 9.53%. C₂₇H₅₈O₇Si₄ requires: C, 53.42; H, 9.63%.
- 30 d) **Methyl 4-trimethylsilyloxy (6,7,13-O-tristrimethylsilyl)monate A** - A solution of methyl diethylphosphonoacetate (0.514ml) in dry 1,2-dimethoxyethane (3ml) was added, dropwise over 5 minutes, to a stirred suspension of sodium hydride (86mg of a 60% dispersion in oil) in dry THF (3ml) under dry argon. After the reaction was complete (hydrogen evolution ceased) the stirred mixture was cooled in

an ice bath and treated, dropwise over 3 minutes, with a solution of the product from Example 3c (more polar isomer, 150mg) in dry 1,2-dimethoxyethane (2ml). The cooling bath was removed and the mixture was stirred for 18 hours. The mixture was treated with saturated ammonium chloride solution (5ml) and extracted with ethyl acetate. The combined organic layers were washed with saturated ammonium chloride solution, dried (MgSO_4), evaporated, and the residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give the title compound (22mg). δ_{H} (CDCl_3) 0.02-0.24 (36H, m, SiCH_3), 0.89 (3H, d, *J* 7.1Hz, 17-H₃), 1.19 (3H, d, *J* 6.3Hz, 14-H₃), 1.40 (1H, m, 12-H), 1.59-1.69 (2H, br.m, 9-H₂), 1.99 (1H, br.m., 8-H), 2.16 (3H, s, 15-H₃), 2.67 (2H, m, 10-H and 11-H), 3.52 (1H, dd, *J* 4.2 and 11.4, 16-H), 3.6-3.78 (2H, m, 5-H and 6-H), 3.78-3.93 (3H, m, 16-H, 13-H and 7-H), 4.28 (1H, d, *J* 2.5Hz, 4-H) and 5.92 (1H, s, 2-H); *m/z* (NH_3^+DCI) 663 (12%, M^+) and 680 (10%, MNH_4^+). (Found M^+ , 662.3533. $\text{C}_{30}\text{H}_{62}\text{O}_8\text{Si}_4$ requires *m/z* 662.3522).

15 **Preparation 4: Ethyl 4-hydroxymononate A** - To a solution of lithium diisopropylamide in THF (4ml) under argon at -70°C, prepared from diisopropylamine (47.7mg, 0.47mmol) and butyl lithium in hexane (0.39ml, 0.47mmol), ethyl 2-trimethylsilylacetate (75mg, 0.47mmol) was added and the mixture stirred at -70°C for 30min. A solution of the more polar ketone (prepared as Preparation 3c) (238.1mg, 0.39mmol) in THF (2.5ml) was added and the mixture stirred at -70°C for 45min. The temperature of the cooling bath was then raised to -50°C and the temperature of the reaction held at -50°C for 1hr. Ammonium chloride solution (1ml) was added and the reaction mixture allowed to warm to room temperature. The solvents were evaporated, ethyl acetate added and the residual water removed. The ethyl acetate solution was dried (anhyd. MgSO_4) and evaporated. The residual oil (334.2mg) was dissolved in THF (15ml) and treated with 0.4M HCl (3ml) for 2min. The reaction mixture was then quenched with sat. sodium bicarbonate solution (3ml) and the solvents evaporated. Ethanol was added, solids filtered off and the filtrate evaporated. This process was repeated several times, using MDC at the final stage. The crude product (130.7mg) was purified by chromatography over silica gel using 2-6% MeOH/MDC as eluant to yield the title compound 53.5mg (35%) as a colourless gum. ν_{max} (KBr) 3420, 2856, 1710, 1653, 1461 and 1220 cm^{-1} ; λ_{max} (EtOH) 217.5nm (ϵ_m 14,231); δ_{H} (CDCl_3) 0.93 (3H, d, *J* 7Hz, 17-H₃), 1.22 (3H, d, *J* 6.2Hz, 14-H₃), 1.27 (3H, t, *J* 5.1Hz, CH_2CH_3), 1.3-3.5 (1H, m, 12-H), 1.63-1.79 (2H, m, 9-H₂), 2.02 (1H, br.m, 8-H), 2.48 (1H, OH), 2.68-1.82 (3H, m, 10-H, 11-H, OH), 2.9-3.02 (2H, OH), 3.62 (1H, d, *J* 11.3Hz, 16-H), 3.68 (1H, dd, *J* 2 and 11.5Hz, 5-H), 3.79 (1H, m, 13-H), 3.86 (1H, m, 6-H), 3.93 (1H, dd, *J* 2.5 and 12Hz, 16-H), 4.02 (1H, br.s, 7-H), 4.16 (2H, q, *J* 7.6Hz,

CH_2CH_3), 4.28 (1H, d, J 8.9Hz, 4-H) and 6.00 (1H, s, 2-H); δ_C (CDCl₃) 12.82 (C-17), 14.35 (CH₂CH₃), 15.97 (C-15), 20.99 (C-14), 31.51 (C-9), 39.55 (C-8), 42.87 (C-12), 55.57 (C-10), 59.86 (C-11), 61.23 (OCH₂), 64.83 (C-6), 65.53 (C-16), 70.57 (C-7), 71.50 (C-13), 73.97 (C-4), 75.88 (C-5), 116.05 (C-2), 158.03 (C-3) and 166.75 (C-1); m/z (NH₃⁺DCI) 389 (48%, MH^+) and 406 (82%, MNH_4^+). Found: MH^+ , 389.2175. C₁₉H₃₂O₈ requires m/z 389.2175.

Preparation 5: Methyl 4-hydroxymonate A (alternative preparation) - To a stirred suspension of sodium hydride (60% in oil) (210mg, 5.28mmol) in dry THF (2ml) under argon, a solution of methyl diethylphosphonate (1.38g, 1.2ml, 6.6mmol) and 15-crown-5 (60mg, 0.26mmol) in dry dimethoxyethane (DME) (2ml) was added over 2min. The resulting pale yellow solution was stirred at room temperature for 20min. then treated with the more polar ketone (prepared as Preparation 3c) (300mg, 0.66mmol) in DME (1.5ml). The reaction mixture was heated slowly to 70°C and after 1hr. 50min. the reaction was quenched with ammonium chloride solution (3ml), extracted into ethyl acetate and dried (anhyd. MgSO₄). Evaporation yielded an oil (500mg) which was dissolved in THF (18ml), treated with 0.4M HCl (4.2ml) for 2min. then quenched with saturated sodium bicarbonate solution (4.2ml). The solvents were removed under vacuum, methanol added, the solids filtered off and the filtrate evaporated. This process was repeated several times and the residual oil chromatographed on silica gel, eluting with 3-5% MeOH/MDC, to yield the title compound (52.1mg, 28%) as a colourless gum. The spectral and chromatographic properties of this compound corresponded to those of the more polar isomer of methyl 4-hydroxymonate A described in Preparation 1e above.

Preparation 6: 7-Methoxycarbonylheptyl-4-hydroxymonate A

25 a) **7-Methoxycarbonylheptyl-2-diethylphosphonoacetate** - A solution of 2-diethylphosphonoacetic acid (2.25g, 11.5mmol) (prepared as described in J.P. Clayton *et al*, J. Chem. Soc. Perkin I 1979, 308), methyl 8-hydroxyoctanoate (2g, 11.5mmol) and dicyclohexylcarbodiimide (2.57g, 11.4mmol) in ethyl acetate (30ml) was stirred at room temperature for 4 hours. The reaction mixture was diluted with 30 ethyl acetate (30ml), filtered, and the filtrate evaporated to a small volume. Toluene (30ml) was added to the residual oil, filtered and the filtrate evaporated to yield the crude title compound (3.45g). This material was purified by flash chromatography over silica gel eluting with toluene, 10% ethyl acetate/hexane and ethyl acetate to yield the title compound (3.26g, 80%) as a colourless oil. ν_{max} (KBr) 2934, 1735, 1270 and 1026cm⁻¹; δ_H (CDCl₃) 1.34 (12H, m, CH₂ and CH₃), 1.5 (4H, m, CH₂), 2.30 (2H, t, J 7.5Hz, CH₂CO₂), 2.96 (2H, d, J 21.7Hz, PCH₂CO₂), 3.66 (3H, s, CO₂CH₃) and 4.15 (6H, m, CH₂O); Found: M^+ , 352.1653. C₁₅H₂₉O₇P requires 352.1651. Found: C, 50.76; H, 7.43. C₁₅H₂₉O₇P requires: C, 50.98; H, 8.27%.

b) **7-Methoxycarbonylheptyl-4-hydroxymonate A** - To a stirred suspension of sodium hydride (60% in oil) (128mg, 3.22mmol) in dry THF (1.5ml) under argon and cooled in ice water, a solution of 7-methoxycarbonylheptyl-2-phosphonoacetate (1.42g, 4.04mmol) in dry THF (1.6ml) was added over 15min. The reaction mixture was stirred slowly for 15min. then the more polar ketone (prepared as Preparation 3c) (244.8mg, 0.4mmol) in dry THF (0.4ml and 1ml syringe wash) over 10min. The reaction mixture was allowed to warm to room temperature, stirred slowly under argon for 18hr. and then poured into 0.1M phosphate buffer at pH 7 containing a little ice. The mixture was extracted with ethyl acetate (3 x 25ml), the combined extracts evaporated to a small volume and ethyl acetate added to the residue. The water layer was removed, the organic layer evaporated and the residue dissolved in THF (15ml). This solution was treated with 0.4M HCl (3ml) for 2min. and then quenched with saturated sodium bicarbonate solution (3ml). After evaporation of the THF, ethyl acetate was added to the residue, the aqueous layer removed and the organic layer dried (anhyd. MgSO₄). Evaporation of the dried solution yielded crude product (1.4g) which was purified by chromatography over silica gel eluting with 3% MeOH/MDC to yield the title compound (100.8mg, 48%) as a colourless gum. ν_{max} (KBr) 3422, 2916, 1735, 1715 and 1215cm⁻¹; λ_{max} (EtOH) 218nm (ϵ_m 14,932); δ_{H} (CDCl₃/D₂O) 0.94 (3H, d, *J* 7Hz, 17-H₃), 1.22 (3H, d, *J* 6Hz, 14-H₃), 1.27-1.43 (7H, m, CH₂ and 12-H), 1.57-1.78 (6H, m, CH₂ and 9-H₂), 2.02 (1H, m, 8-H), 2.15 (3H, s, 15-H₃), 2.30 (2H, t, *J* 7.5Hz, 2'-H₂), 2.73 (1H, dd, *J* 2 and 10Hz, 11-H), 2.79 (1H, ddd, *J* 2, 5 and 6.5Hz, 10-H), 3.63 (1H, d, *J* 11.5Hz, 16-H), 3.66 (3H, s, 15-H₃), 3.68 (1H, dd, *J* 2 and 9.5Hz, 5-H), 3.79 (1H, m, 13-H), 3.86 (1H, dd, *J* 3.5 and 9.5Hz, 6-H), 3.92 (1H, dd, *J* 3 and 11.5Hz, 16-H), 4.01 (1H, t, *J* 3Hz, 7-H), 4.13 (2H, dt, *J* 1 and 7Hz, 8'-H), 4.28 (1H, t, *J* 2.5Hz, 4-H) and 6.00 (1H, s, 2-H); δ_{C} 12.82 (C-17), 16.0 (C-15), 21.0 (C-14), 24.9, 25.89, 28.67, 28.92 and 29.04 (chain CH₂), 31.60 (C-9), 34.11 (C-2'), 39.61 (C-8), 42.85 (C-12), 51.54 (OCH₃), 55.67 (C-10), 61.16 (C-11), 64.05 (C-8'), 64.77 (C-6), 65.63 (C-16), 70.85 (C-7), 71.40 (C-13), 73.88 (C-4), 76.02 (C-5), 116 (C-2), 158.2 (C-3), 167.0 (C-1) and 174.42 (C-1'); FAB (glycerol). Found: M⁺ 517.2994. C₂₆H₄₄O₁₀ requires *m/z* 517.3013.

Preparation 7: 7-Methoxycarbonylheptyl 4-hydroxymonate C - A stirred solution of 7-methoxycarbonylheptyl 4-hydroxymonate A 340mg, 0.66mmol) (prepared as Preparation 6 above) in dichloromethane (10ml) under argon was treated with pyridine (0.319ml, 320mg, 3.95mmol) and then cooled in ice. Trifluoroacetyl chloride (0.37ml, 600mg, 3.29mmol) was added, the mixture stirred for 1hr then partitioned between dichloromethane (15ml) and 10% sodium bicarbonate solution (15ml). The organic layer was washed with 10% sodium bicarbonate solution, 10%

citric acid solution, brine and dried (anhyd. MgSO₄) to yield 667.3mg of the crude tetrachloroacetyl derivative of 7-methoxycarbonylheptyl 4-hydroxymonate A. This material was then deoxygenated to the monate C derivative as follows. To dry sodium iodide (900mg, 6mmol) in stirred anhydrous acetonitrile (4ml) under argon 5 trifluoroacetic anhydride (0.256ml, 379mg, 1.8mmol) was added and the mixture stirred for a further 5min. then cooled in ice. The tetrachloroacetyl derivative prepared above was then added in anhydrous acetonitrile (2ml) over 3min. The ice bath was removed after 10min. and the solution stirred under argon in the dark for 18hr. The reaction mixture was then poured into ether (25ml) and neutral sodium 10 sodium metabisulphite solution (25ml). The organic layer was washed further with neutral sodium metabisulphite solution, sodium bicarbonate solution and brine, then dried (anhyd. MgSO₄). Evaporation of the ether yielded 614mg of material which was dissolved in methanol (10ml) stirred with anhyd. potassium carbonate (70mg) for 1hr, filtered and evaporated. Chromatography of the residue over silica gel eluting with 3- 15 5% MeOH/MDC yielded the title compound (175.4mg, 62%) as a colourless gum.
 ν_{max} (KBr) 3413, 2930, 1738, 1717, 1633, 1215, 1153 and 1060cm⁻¹; λ_{max} 218.5 (ϵ_{m} 14,235) and 201.5 (ϵ_{m} 11,246)nm; δ_{H} (CDCl₃/CD₃OD) 0.93 (3H, d, *J* 7Hz, 17-H₃), 1.08 (3H, d, *J* 6Hz, 14-H₃), 1.2-1.37 (6H, m, CH₂), 1.48-1.64 (4H, m, CH₂), 1.76 (1H, m, 8-H), 2.07 (3H, s, 15-H₃), 1.98-1.22 (3H, m, 9-H₂ and 12-H), 2.25 (2H, 20 t, *J* 7.5Hz, 2'-H₂), 3.45-3.52 (2H, m, 13-H and 16-H), 3.58 (1/2H, d, *J* 2Hz, 1/2 6-H, other doublet obscured by CO₂Me), 3.60 (3H, s, CO₂Me + 1/2 6-H), 3.74 (1H, dd, *J* 3 and 9Hz, 16-H), 3.77 (1H, dd, *J* 3 and 10Hz, 5-H), 3.86 (1H, t, *J* 3Hz, 7-H), 4.02 (2H, t, *J* 7Hz, 8'-H), 4.22 (1H, s, 4-H), 5.38 (2H, m, 10-H and 11-H) and 5.96 (1H, t, *J* 1.4Hz, 2-H); δ_{C} (CDCl₃/CD₃OD) 15.46 (C-17), 16.13 (C-15), 19.79 (C- 25 14), 24.37, 25.35, 28.14, 28.39 and 28.51 (chain CH₂), 31.67 (C-9), 33.60 (C-2'), 41.06 (C-8), 43.99 (C-12), 51.06 (OCH₃), 63.51 (C-8'), 63.87 (C-6), 64.77 (C-16), 69.61 (C-7), 70.72 (C-13), 73.06 (C-4), 75.05 (C-5), 115.07 (C-2), 128.53 (C-10), 134.01 (C-11), 158.28 (C-3), 166.64 (C-1) and 174.23 (C-1'). FAB (glycerol) Found: MH^+ 501.3064. C₂₆H₄₄O₉ requires *m/z* 501.3064.

30 **Preparation 8: 7-Carboxyheptyl 4-hydroxymonate A** - To a solution of Subtilisin Carlsberg (25mg) in 0.1M phosphate buffer at pH 7 (90ml) a solution of 7-carboxymethylheptyl 4-hydroxymonate A (94.3mg, 0.18mmol) in acetone (10ml) was added and the solution stirred at room temperature for 48hr. The volume was reduced to *ca*.60ml, ethyl acetate added (20ml), and with vigorous stirring the pH of the 35 aqueous layer was adjusted to 3 with 0.4M phosphoric acid. The mixture was partitioned with MDC, filtered, the material on the filter washed with ethyl acetate and the aqueous filtrate washed with ethyl acetate (3 x 50ml). The combined organics were dried (anhyd. MgSO₄) to yield the title compound (78.6mg, 81%) as a

colourless gum. ν_{max} (KBr) 2929, 1712, 1652 and 1242cm⁻¹; λ_{max} 218.5 (ε_m 13,295)nm; δ_H (CDCl₃) 0.95 (3H, d, *J* 1 Hz, 17-H₃), 1.20 (3H, d, *J* 6.6Hz, 14-H₃), 1.29-1.5 (7H, m, CH₂ and 12-H), 1.5-1.85 (6H, m, CH₂ and 9-H₂), 1.85-1.98 (1H, m, 8-H), 2.12 (3H, s, 15-H₃), 2.28 (2H, t, *J* 7.4Hz, 2'-H₂), 2.73 (1H, dd, *J* 2.2 and 7.6Hz, 11-H), 2.82 (1H, dt, *J* 2 and 6Hz, 10-H), 3.59 (1H, d, *J* 11.3Hz, 16-H), 3.69 (1H, dd, *J* 1.7 and 9.6Hz, 5-H), 3.73-3.9 (3H, m, 13-H, 6-H and 16-H), 3.96 (1H, t, *J* 3Hz, 7-H), 4.09 (2H, t, *J* 6.4Hz, 8'-H₂), 4.32 (1H, br.s, 4-H) and 6.05 (1H, t, *J* 1.2Hz, 2-H); m/z (NH₃⁺DCI) 503 (14%, M⁺) and 520 (10%, MNH₄⁺)

Preparation 9: 7-Carboxyheptyl 4-hydroxymonate C - A solution of Subtilisin Carlsberg (670mg in 3ml water at pH 7) was sealed in a short piece of viscose tubing then put into a stirred solution of 7-carboxymethylheptyl 4-hydroxymonate C in water (160ml) and acetone (16ml) at pH 7. The stirred reaction mixture was maintained at pH 7 with 0.01M NaOH on a Metrohm Autotitration apparatus overnight. After removal of the bag of enzyme, the solution was concentrated to ca. 100ml, the pH adjusted to 3 with 0.1M HCl and the solution partitioned with MDC. The mixture was filtered to remove an emulsion and the filter washed with ethyl acetate. The aqueous filtrate was extracted further with ethyl acetate (2 x 30ml) and the combined organic fractions dried (anhyd. MgSO₄) to yield the title compound (132.6mg, 85%) as a colourless gum. δ_H (CDCl₃/CD₃OD) 0.87 (3H, d, *J* 6.8Hz, 17-H₃), 1.01 (3H, d, *J* 6.3, 14-H₃), 1.1-1.38 (6H, m, CH₂), 1.38-1.62 (4H, m, CH₂), 1.62-1.75 (1H, m, H-8), 1.9-2.23 (8H, m, 15-H₃, 12-H, 9-H₂ and 2'-H₂), 3.46-3.59 (2H, m, 16-H and 13-H), 3.63 (1H, d, *J* 2.8 and 6.8Hz, H-6), 3.7-3.83 (2H, m, 5H and 16-H), 3.85 (1H, t, *J* 1Hz, 7-H, 4.05 (2H, t, *J* 6.1Hz, 18'-H), 4.25 (1H, s, 4-H), 5.31-5.5 (2H, m, 10-H and 11-H) and 6.01 (1H, s, 2-H).

Example 1: 6-[4-{5S-(2S,3S-Epoxy-5S-hydroxy-4S-methyl-hexyl)-3R,4R-dihydroxytetrahydropyran-2S-yl}]-4-hydroxy-3-methyl-but-2E-enoyloxyoctanoylamino]-1,2-dithiolo-[4,3-b]pyrrol-5(4H)-one - To a stirred solution of the acid prepared as in Preparation 8 (67.5mg, 0.13mmol) and triethylamine (15mg, 2μl, 0.148mmol) in dry tetrahydrofuran (2ml) under argon at -5°C, isobutyl-chloroformate (18.13mg, 17μl, 0.134mmol) was added and the solution stirred for 30min. Further quantities of triethylamine (5μl) and isobutyl chloroformate (4μl) were added and after stirring for 10min, triethylamine (30mg, 42μl, 0.30mmol) and 6-amino-1,2-dithiolo[4,3-b]pyrrol-5(4H)-one hydrochloride (35mg, 0.168mmol) were added. After 1hr tetrahydrofuran was added (1ml) and the mixture stirred at room temperature for 24hr. The reaction mixture was evaporated to dryness and partitioned between water (10ml) and MDC (10ml). A brown precipitate was filtered off and set aside. The organic phase was removed, the aqueous layer extracted with ethyl acetate

(2 x 20ml) and the combined organic layers dried (anhyd. MgSO₄). The brown precipitate was dissolved in ethyl acetate and dried (anhyd. MgSO₄). The combined dried solutions were evaporated and the residue absorbed onto silica gel using methanol. The silica was placed on a column of silica gel (2.4g) made up in MDC and then eluted with 5-10% MeOH/MDC to yield the title product (20mg, 23%); ν_{max} (KBr) 3435, 2865, 1645, 1527, 1212 and 1050cm⁻¹; λ_{max} (EtOH) 214.5 (ϵ_m 28,544), 302.5 (ϵ_m 7,111) and 391 (ϵ_m 14.823)nm; δ_H (CD₃OD) 0.94 (3H, d, *J* 7Hz, 17-H₃), 1.20 (3H, d, *J* 6.4Hz, 14-H₃), 1.32-1.48 (7H, m, CH₂ and 12-H), 1.58-1.72 (5H, m, CH₂ and 9-H₂), 1.78 (1H, m, 9-H), 1.92 (1H, m, 8-H), 2.12 (3H, s, 15-H₃), 2.39 (2H, t, *J* 7.3Hz, 2'-H₂), 2.72 (1H, dd, *J* 4 and 6Hz, 11-H), 2.81 (1H, m, 10-H), 3.59 (1H, dt, *J* 1 and 11Hz, 16-H), 3.69 (1H, dd, *J* 2 and 7.5Hz, 5-H), 3.78 (1H, dq, *J* 5 and 6.5Hz, 13-H), 3.82 (1H, dd, *J* 3 and 9Hz, 6-H), 3.85 (1H, dd, *J* 3 and 11Hz, 16-H), 3.95 (1H, t, *J* 3Hz, 7-H), 4.09 (2H, m, 8'-H₂), 4.32 (1H, t, *J* 2Hz, 4-H), 6.04 (1H, t, *J* 1Hz, 2-H) and 7.05 (1H, s, 3"-H); *m/z* (electrospray) 657 (60%, H⁺); FAB (glycerol) Found: MH⁺ 657.2516. C₃₀H₄₄N₂O₁₀S₂ requires *m/z* 657.2516.

The configuration of the C-4 hydroxyl group is considered to be the same as that in thiomarinol, given that thiomarinol is also obtainable from the same precursor (as described in preparation

20 **Example 2: 6-[4-{5S-(5S-Hydroxy-4R-methyl-hex-2E-enyl)-3R,4R-dihydroxytetrahydropyran-2S-yl}-4-hydroxy-3-methyl-but-2E-enoyl-oxy-octanoylamino]-1,2-dithiolo-[4,3-b]pyrrol-5(4H)-one (thiomarinol) -** In a similar manner to Example 1 above, the title compound was synthesised from the acid prepared in Preparation 9 (132.6mg, 0.27mmol) in 51% yield (89.5mg); m.p. 103-104°C (MeOH); Found: C, 54.43; H, 6.81; N, 4.19; S, 10.19% C₃₀H₄₄N₂O₉S₂.2MeOH requires C, 54.1; H, 7.43; N, 3.97; S, 9.1% ν_{max} (KBr) 3403, 3053, 2928, 1645, 1525, 1216, 1151 and 1061cm⁻¹; λ_{max} (MeOH) 213.5 (ϵ_m 26,287), 301.5 (ϵ_m 3,626), and 385.5 (ϵ_m 12,791)nm; δ_H (CDCl₃/CD₃OD) 0.92 (3H, d, *J* 6.8Hz, 17-H₃), 1.07 (3H, d, *J* 6.3Hz, 14-H₃), 1.30 (6H, br.s, CH₂), 1.50-1.70 (4H, m, CH₂), 1.75 (1H, m, 8-H), 1.57 (3H, s, 15-H₃), 1.8-2.2 (3H, m, 9-H₂ and 12-H), 2.29 (2H, t, *J* 7.2Hz, 2'-H₂), 3.40-3.56 (2H, m, 13-H and 16-H), 3.6 (1H, dd, *J* 2 and 9.7Hz, 6-H), 3.73-3.78 (2H, m, 5-H and 16-H), 3.86 (1H, t, *J* 3.2Hz, 7-H), 4.24 (1H, t, *J* 2Hz, 4-H), 5.37 (2H, m, 10-H and 11-H), 5.98 (1H, s, 2-H) and 6.99 (1H, s, 3"-H). *m/z* (electrospray) 639 (100% M-H⁻); FAB (glycerol) Found: MH⁺ 641.2567. C₃₀H₄₄N₂O₉S₂ requires *m/z* 641.2567.

The ¹H nmr of this material was identical to natural thiomarinol and both compounds co-eluted on hplc; C₁₈ column, eluant 64% MeOH in 0.05M ammonium acetate buffer pH 4.5, retention time 3.2min., detection at 390nm.

Example 3: 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide A: isomer A

- a) **4-Hydroxymonic acid A : Isomer A** - The less polar product of preparation 1e in trimethyl orthoformate is treated with a catalytic amount of toluene *p*-sulphonic acid. After 0.5h the solvent is removed and the oil is immediately dissolved in 1N sodium hydroxide. After 3h at 65°C the solution is cooled and the pH adjusted to 7.0 with concentrated hydrochloric acid. Methanol is added and the pH adjusted to 2.0 with 5N hydrochloric acid. After 0.25h, the pH is raised to and maintained at 9-9.5 with sodium hydroxide solution for 3h, (hplc analysis indicates that reaction 10 complete). The pH is readjusted to 7.0. The solution is evaporated to low volume, saturated with sodium chloride, layered with ethyl acetate and acidified to pH 3 with stirring. The organic phase is separated, dried (MgSO₄) and evaporated to afford the title compound.
- b) **Sodium 4-hydroxymonate A: Isomer A** - The product from example 3a in 15 water is treated carefully with portions of solid sodium hydrogen carbonate until a pH of 7 is obtained. The solution is evaporated to low volume and freeze dried to give the title compound.
- c) **Methyl 4-hydroxypseudomonate A: Isomer A** - The product from example 3b and methyl 9-bromononanoate (1.3 to 2 equivalents) in DMF is heated at 70°C for 20 2 hours (tlc. monitoring of reaction). The mixture is cooled, evaporated to low volume, and treated with ethyl acetate and sodium bicarbonate solution. The organic phase is separated, washed with brine, dried (MgSO₄) and evaporated. The residue is chromatographed on silica eluting with dichloromethane/methanol mixtures to give the title compound.
- d) **4-Hydroxypseudomonic acid A: Isomer A** - A solution of the product from example 3c in acetone (0.1ml/mg) is diluted with 0.1M disodium hydrogen phosphate (1ml/mg; adjusted to pH 7.0 with concentrated hydrochloric acid) and treated with Subtilisin Carlsberg Protease (0.5 equivalents). After 24 hours at room temperature (monitoring by tlc and hplc), the solution is evaporated to low volume, layered with 30 ethyl acetate and acidified to pH 3 with stirring. The organic phase is separated, dried (MgSO₄) and evaporated to give the title compound.
- e) **4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide A: Isomer A** - A stirred solution of the product of example 3d in THF at -10°C is treated sequentially with triethylamine (2 equivalents) and isobutyl 35 chloroformate (1 equivalent). After 20 minutes 6-amino-4-methyl-1,2-dithiolo-[4,3-b]pyrrol-5-(4H)-one hydrochloride (1 equivalent) is added. The mixture stirred at room temperature and the reaction progress monitored (tlc/hplc). When reaction is complete, the reaction mixture is diluted with ethyl acetate, washed with water,

saturated sodium hydrogen carbonate, and brine, then dried ($MgSO_4$) and evaporated. The residue is chromatographed on silica eluting dichloromethane/methanol mixtures to give the title compound.

Example 4 - 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5-(4H)-oxopyrrol-6-yl)pseudomonamide A: Isomer B

- 5 a) 4-Hydroxymonic acid A: Isomer B - Using the procedure described in example 3a the more polar product of preparation 1e is converted to the title compound.
- 10 b) Sodium 4-hydroxymonate A: Isomer B - Using the procedure described in example 3b the product from example 4a is converted to the title compound.
- c) Methyl 4-hydroxypseudomonate A: Isomer B - Using the procedure described in example 3c the product from example 4b is converted to the title compound.
- 15 d) 4-Hydroxypseudomonic acid A: Isomer B - Using the procedure described in example 3d the product from example 4c is converted to the title compound.
- e) 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide A: Isomer B - Using the procedure described in example 3e, the product from example 4d is converted to the title compound.

Example 5 - 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide C: Isomer A

- 20 a) Methyl 4-hydroxypseudomonate C: Isomer A - The product from example 3c in dichloromethane and pyridine (5.5 equivalents) is treated dropwise with trichloroacetyl chloride (5 equivalents). After 1h the mixture is washed with aqueous sodium bicarbonate, dilute citric acid and brine, then dried ($MgSO_4$) and evaporated to crude acylated material.

Trifluoroacetic anhydride (1 equivalent) is added to a mixture of sodium iodide (3.3 equivalents) and acetonitrile at 20°C. After 5min. a solution of the above acylated material in acetonitrile is added and the mixture is stirred for 18h. Diethyl ether is added and the mixture washed with aqueous sodium hydrogen sulphite, aqueous sodium hydrogen carbonate and brine, then dried ($MgSO_4$) and evaporated. The residue is then dissolved in ethanol and potassium carbonate (8 equivalents) is added. After 2h (tlc monitoring) ethyl acetate is added and the mixture washed with water, brine then dried ($MgSO_4$) and evaporated. The residue is chromatographed on silica eluting methanol/dichloromethane mixtures to give the title compound.

- 25 b) 4-Hydroxypseudomonate C: Isomer A - Using the procedure described in example 3d the product from example 5a is converted to the title compound.
- c) 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide C: Isomer A - Using the procedure described in example 3e the

product from example 5b is converted to the title compound.

Example 6 - 4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide C: isomer B

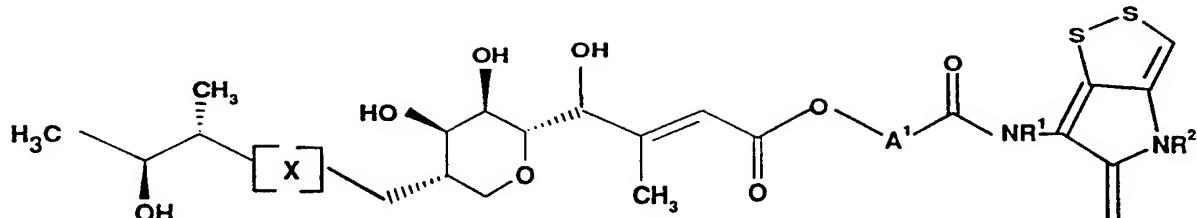
- 5 a) **Methyl 4-hydroxypseudomonate C: Isomer B** - Using the procedure described in example 5a, the product from example 4c is converted to the title compound.
- 10 b) **4-Hydroxypseudomonate C: Isomer B** - Using the procedure described in example 3d the product from example 6a is converted to the title compound.
- 10 c) **4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide C: Isomer B** - Using the procedure described in example 3c the product from example 6b is converted to the title compound.

Example 7 - Pharmaceutical Formulation

Compound of formula (I)	2%
polyethylene glycol 400	59
polyethylene glycol 4000	39

Claims

1. A compound of formula (I):



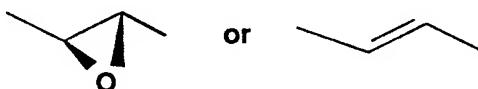
5

(I)

in which A^1 is a group of atoms for linking $C(O)O$ with $CONR^1$;

10 R^1 and R^2 , which may be the same or different, is each selected from hydrogen or (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl, (C_{2-6}) alkenyl, aryl, aryl(C_{1-4})alkyl or heterocyclyl, each of which may be optionally substituted;

X is an epoxy moiety or an E-double bond moiety:



15

and excluding the compound named "thiomarinol".

2. A compound as claimed in claim 1 in which A^1 is $C(R^3)(R^4)$; $[C(R^3)(R^4)]_m A^2$; $[C(R^3)(R^4)]_m A^2 A^3$; $[C(R^3)(R^4)]_m A^3$; or $[C(R^3)(R^4)]_m A^3 A^2$;

20 in which:

m is 0 or 1;

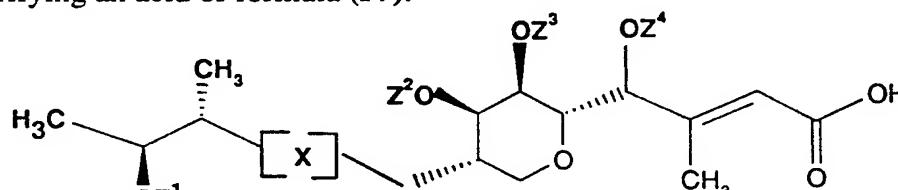
R^3 and R^4 , which may be the same or different, is each selected from hydrogen or (C_{1-6}) alkyl;

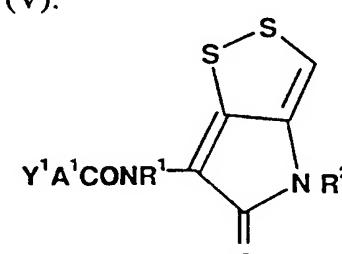
A^2 is a (C_{3-7}) cycloalkylene group, an optionally substituted aryl group, preferably 25 phenylene, or an optionally substituted heterocyclyl group; and

A^3 is a polymethylene chain having between 1 and 20 carbon atoms, preferably 4 and 9 carbon atoms, which chain may be optionally substituted, for instance by a (C_{1-6}) alkyl group, and which chain may be optionally interrupted at one or more places by a moiety M in which:

30 M is a chain of one or more atoms for linking two polymethylene chains and which may be the same or different if there is more than one interruption.

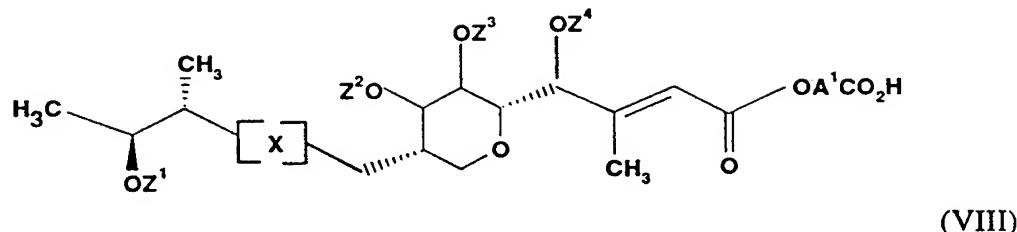
3. A compound as claimed in claim 1 or 2 in which A¹ is an optionally substituted polymethylene chain (CH₂)_n in which n is an integer from 1 to 10.
4. A compound as claimed in claim 3 in which n is from 5 to 9.
5
5. A compound as claimed in claim 4 in which n is 6, 7 or 8.
6. A compound as claimed in any one of the preceding claims in which R¹ is hydrogen or (C₁₋₆)alkyl.
10
7. A compound as claimed in claim 6 in which R¹ is hydrogen.
8. A compound as claimed in any one of the preceding claims in which R² is hydrogen or (C₁₋₆)alkyl.
15
9. A compound as claimed in claim 8 in which R² is hydrogen.
10. A compound of formula (I) as defined in claim 1 selected from:
6-[4-{5S-(2S,3S-Epoxy-5S-hydroxy-4S-methyl-hexyl)-3R,4R-
20 dihydroxytetrahydropyran-2S-yl}]-4-hydroxy-3-methyl-but-2E-enoyl-
oxyoctanoylamino]-1,2-dithiolo-[4,3-b]pyrrol-5(4H)-one;
4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-yl)pseudomonamide
A: Isomer A;
4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5-(4H)-oxopyrrol-6-
25 yl)pseudomonamide A: Isomer B;
4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-
yl)pseudomonamide C: Isomer A; and
4-Hydroxy-N-(4-methyl-1,2-dithiolo-[4,3-b]-5(4H)-oxopyrrol-6-
yl)pseudomonamide C: Isomer B.
30
11. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1 and a pharmaceutically acceptable carrier or excipient.
12. A compound of formula (I) as defined in claim 1 for use in therapy.
35
13. The use of a compound of formula (I) as defined in claim 1 in the manufacture of a medicament for treating infections caused by mycoplasma.

14. The use of a compound of formula (I) as defined in claim 1 or thiomarinol in the manufacture of a medicament for treating fungal infections.
15. The use of a compound of formula (I) as defined in claim 1 in which X is an E-
5 double bond in the manufacture of a medicament for treating bacterial infection caused by *Pseudomonas* sp..
16. A herbicidal composition comprising a compound of formula (I) as defined in claim 1 or thiomarinol and a herbicidally acceptable carrier.
- 10 17. A process of severely damaging or killing unwanted plants which process comprises applying to the plants or the growth medium of the plants a herbicidally effective amount of a compound of formula (I) as defined in claim 1 or thiomarinol.
- 15 18. A process for preparing a compound of formula (I) as defined in claim 1 or thiomarinol which process comprises:
(a) esterifying an acid of formula (IV):
- 

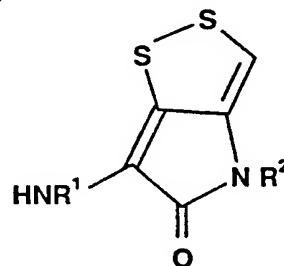
(IV)
- 20 in which X is as defined in claim 1 and Z¹, Z², Z³ and Z⁴, which may be the same or different, is each hydrogen or a hydroxyl protecting group; or a salt or an activated derivative thereof:
with a compound of formula (V):
- 

(V)
- 25 in which:
Y¹ is a reactive esterifying leaving group; and
A¹, R¹ and R² are as hereinbefore defined;
under ester forming conditions; or

(b) reacting an acid of formula (VIII):



- 5 in which A^1 , X , Z^1 , Z^2 , Z^3 and Z^4 are as hereinbefore defined;
or an activated derivative thereof;
with an amine of formula (VII):



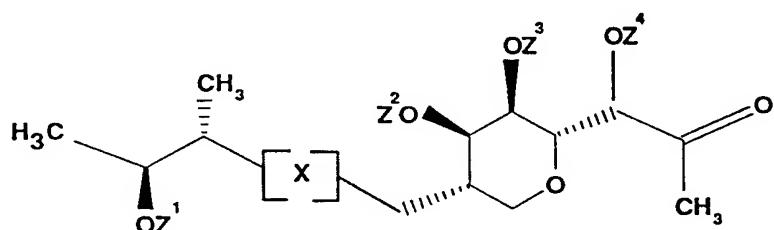
- 10 in which R^1 and R^2 are as hereinbefore defined;
under amide forming conditions; and thereafter, and if necessary, removing any
hydroxyl protecting groups.

15 19. A compound of formula (IV) as defined in claim 18 or a (C₁₋₆)alkyl ester
thereof.

20. A compound of formula (V) as defined in claim 18.

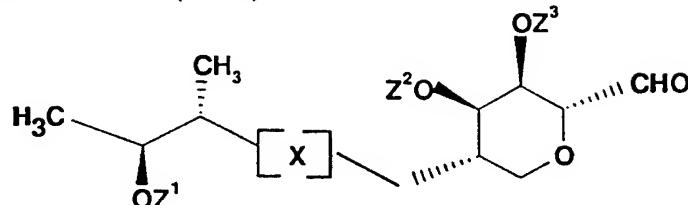
21. A compound of formula (VIII) as defined in claim 18 or a (C₁₋₆)alkyl ester
20 thereof.

22. A compound of formula (XI):



in which X, Z¹, Z², Z³ and Z⁴ are as defined in claim 20.

23. A compound of formula (XVII):



(XVII)

in which X, Z¹, Z² and Z³ are as defined in claim 18.

24. A compound selected from:

- Methyl 4-hydroxymonate A;
 10 Ethyl 4-hydroxymonate A;
 7-Methoxycarbonylheptyl 4-hydroxymonate A;
 7-Methoxycarbonylheptyl 4-hydroxymonate C;
 7-Carboxyheptyl 4-hydroxymonate A;
 7-Carboxyheptyl 4-hydroxymonate C;
 15 3R,4R-Dihydroxyoxy-5S-(2S,3S-epoxy-4S-methyl-5S-hydroxyhexyl)-2R-(2-oxo-1-hydroxyprop-1-yl)tetrahydro-pyran;
 2(R),3(R),4(R),5(S)-2-Formyl-3,4-dihydroxy-5-(2S,3S-epoxy-5S-hydroxy-4S-methylhexyl)tetrahydropyran;
 and the corresponding hydroxyl-protected derivatives.
- 20 25. A compound according to claim 1 substantially as hereinbefore described with reference to any one of the Examples.

INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/EP 94/01582

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07D495/04 C07D309/10 A01N43/90 A61K31/40 /
(C07D495/04, 339:00, 209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07D A01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF ORGANIC CHEMISTRY. vol. 51, no. 20, 1986, EASTON US pages 3916 - 3918 D. R. WILLIAMS ET AL 'Tozal synthesis of (+)-pseudomonic acid C' see page 3917, compound 15 ---	23
X	EP,A,0 512 824 (SANKYO) 11 November 1992 cited in the application see claims 1,7 ---	1,13,15
X	DE,A,36 02 148 (IMPERIAL CHEMICAL INDUSTRIES) 7 August 1986 see claim 1; table 1 ---	20

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

26 July 1994

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INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/EP 94/01582

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	JOURNAL OF ANTIBIOTICS. vol. 46, no. 12, December 1993, TOKYO JP pages 1834 - 1842 H. SHIOZAWA ET AL 'Thiomarinol, a new hybrid antimicrobial antibiotic produced by a marine bacterium' cited in the application see the whole document -----	1, 13-15, 19, 20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/01582

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0512824	11-11-92	AU-B-	646615	24-02-94
		AU-A-	1603392	12-11-92
		CN-A-	1067921	13-01-93
		JP-A-	5132486	28-05-93
		NZ-A-	242648	25-03-94
		US-A-	5292892	08-03-94
DE-A-3602148	07-08-86	AU-A-	5245986	07-08-86
		FR-A-	2590573	29-05-87
		GB-A-	2173499	15-10-86
		JP-A-	61183290	15-08-86